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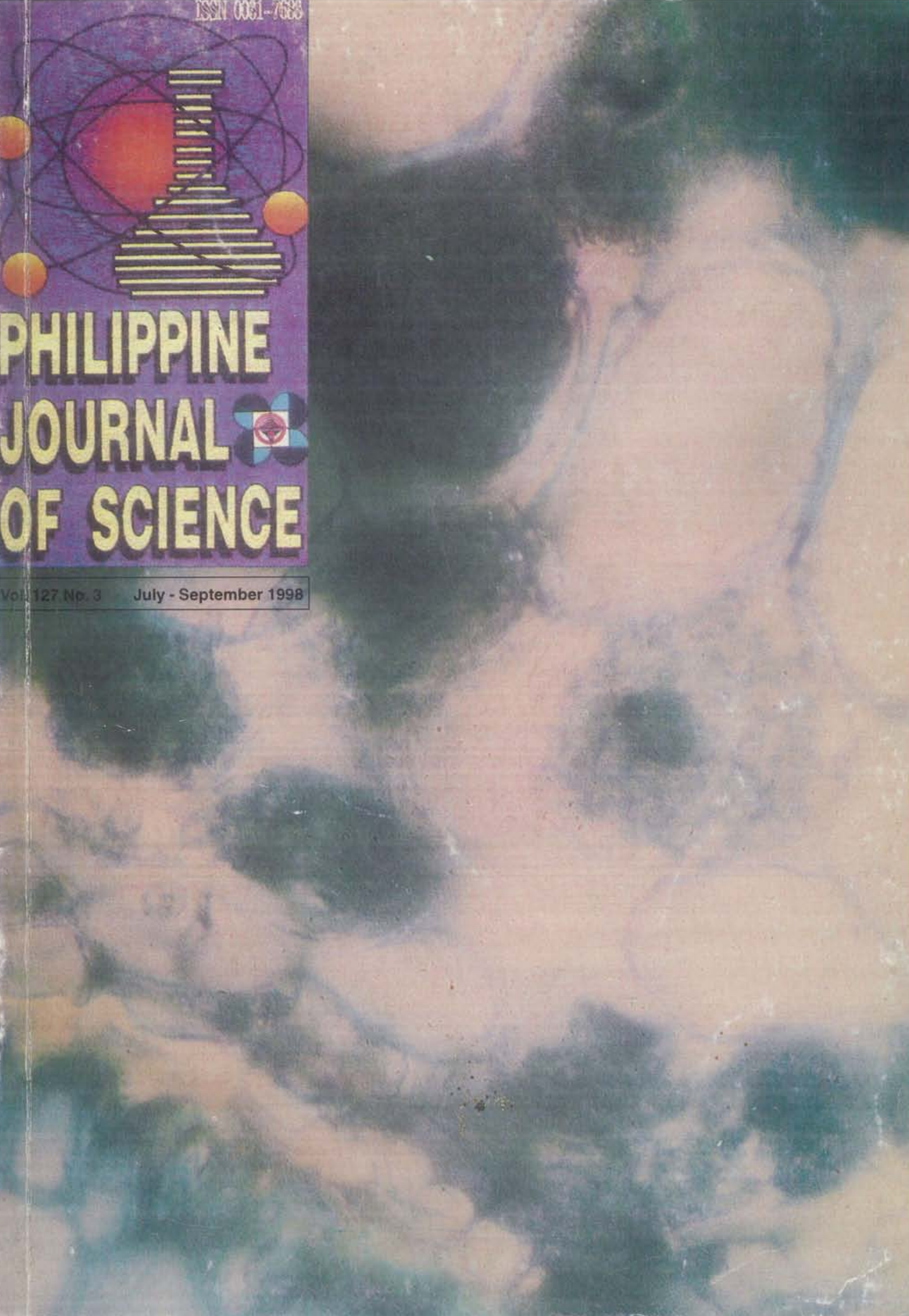

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Optimum Amplification and Frequency Shifts of Light by Drift-Type Photorefractive Crystal

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ABSTRACT

Based on Kukhtarev's material equations for photorefractive crystals and assuming two-wave mixing configuration, an equation for the modulation of the index of refraction is derived. The steady state amplification properties of a weak signal by a drift-type photorefractive crystal are studied numerically. The numerical results are in agreement with experimental measurements using a bismuth silicon oxide (BSO) crystal.

INTRODUCTION

Light-induced changes in the index of refraction was first observed in lithium niobate in 1968 [1] and was initially deemed to cause detrimental effects on the nonlinear operations of the crystal. Two years later this phenomenon, now referred to as photorefractivity, has been suggested to have potential applications in holographic storage [2]. Since then photorefractive crystals (PRCS) have been studied extensively, particularly for their applications in optical storage, optical computing, real-time interferometry, image amplification and image processing . They have also been increasingly popular in physics teaching laboratories since they exhibit easily observable phenomena in nonlinear optics (such as phase conjugation) [3-4]. For many applications, the significant optical process is the formation of a grating by two coherent light beams. This process referred to as two-wave mixing can result to amplication of weak optical signal and has opened possibility for the use of a PRC as a gain medium in an optical oscillator. Photorefractive oscillators (PROS) have been shown to exhibit complex spatio-temporal patterns which make them good candidates for the study of turbulence[5].

The basic theoretical foundation for the study of grating formation in photorefractive crystals was laid down by Kukhtarev [6]. Two coherent beams interfere to form alternating bright and dark regions in the crystal. In the bright regions, light ionizes the molecules which results to the diffusion or drifting of the charge carriers to the dark regions where they are trapped by the impurities of the crystal. The separation of charges result to a space charge electric field which then induces a modulation of the index of refraction by linear electro-optic effect. The gain coefficient (which is measure of light amplification) is determined by the amplitude of the index grating and to some extent the phase shift of the grating from the interference pattern. Diffusion-type crystals generally have large gain coefficients (because of their large electro-optic coefficients) and are common materials used as phase conjugating mirrors (PCMs). In these crystals, optimum gain occurs when the grating phase shift is $\pi/2$. Other crystals with smaller electro-optic coefficients, however, can attain large gain coefficients only in the presence of an external electric field applied perpendicular to the optical axis. These crystals which are referred to as drift-type crystals, have gain which are more complicated than that of the diffusion-type. For example, unlike a diffusion-type crystal whose optimum gain corresponds to a stationary grating, a drift-type crystal has optimum gain for a nonstationary grating, the velocity of which depends on various factors such as the external field, grating wavelength and the intensity of the strong light beam.

Several studies have been reported on gain properties of PRCs. Under assumptions appropriate for diffusion-type crystals, the simplified solutions of Kukhtarev equations have been done. For drift-type crystals, Huignard derived an expression for the optimum gain [7]. This as well as the later work by Refrieger [8], provided an intuitive insight on grating formation and the factors that determine optimum gain for this type. However, certain assumptions which have been made in the derivation of the analytical expressions for the optimum gain and the corresponding grating velocity may not necessarily apply under the usual experimental conditions. In this paper, a general equation for the index of refraction is derived from Kukhtarev's material equations [6]. Since no assumptions were made on the values of relevant material parameters, the equation applies both to diffusion and drift-type photorefractive crystals and accounts for the effect of controllable parameters such as the externally applied field. Using the material properties of bismuth silicon oxide, the dependence of the optimum gain and frequency shift on the external field and grating wavelength is explored and compared with the previously reported results based on some approximations [7,8]. The results of the experiments are also presented and compared with those obtained from the theory.

Theory

A. Equation for the Index of Refraction

The material equations describing the grating formulation in a photorefractive crystal [6] are:

$$\begin{aligned} e\frac{\partial N_D^+}{\partial t} &= e\frac{\partial n}{\partial t} + \nabla \cdot \mathbf{J} && (1) \\ \frac{\partial N_D^+}{\partial t} &= (\beta_e + sI)\left(N_D - N_D^+\right) - \gamma_R n N_D^+ && (2) \\ \mathbf{J} &= e\mu n \mathbf{E}_s - k_B T \mu \nabla n && (3) \\ \nabla \cdot (\varepsilon_o \varepsilon_s \mathbf{E}_s) &= e(n + N_A - N_D) && (4) \end{aligned}$$

The first equation is the continuity equation describing the dependence of the flow of current, J, on the rate of change in the ionized donor density, N_D^+, and electron density, n. The second equation is the rate equation for N_D^+, where I is the light intensity, β_e is the rate of thermal generation, s is the cross-section of photoionization and γ_R is the recombination rate. The third equation expresses the contribution to the current density of the drifting of the electrons in the presence of the static electric field, $\mathbf{E}_s$. In equation 3, μ is the mobility and T is the temperature. The photovoltaic contribution (associated with crystal anisotropy) which is very small in many nonlinear crystals is neglected. The fourth equation is simply the Poisson's equation where ε_s is the permittivity of the crystal and N_A is the acceptor density.

The coherent light beams are assumed to have the same directions of polarization and beam sizes which are large compared to the cross-sectional area of the crystal. Thus it can be assumed that the transverse profile of the beams are essentially uniform in the crystal and that the complex amplitudes of the fields are functions only to z (where the z-axis is as shown in figure 1). This is known in the literature as the place wave-approximation which allows the electric field to be written in the following form:

$$\begin{aligned} E_1(z,t) &= \mathcal{E}_1(z,t)\exp\left[i\left(\mathbf{k}_1 \cdot \mathbf{r} - \omega_1 t\right)\right] + \text{c.c.} && (5) \\ E_2(z,t) &= \mathcal{E}_2(z,t)\exp\left[i\left(\mathbf{k}_2 \cdot \mathbf{r} - \omega_2 t\right)\right] + \text{c.c} && (6) \end{aligned}$$

The intensity of the light in the crystal is therefore given by:

$$I(x,z,t) = I_o(z,t) + \{\mathcal{E}_1\mathcal{E}_2^*(z,t)\exp\left[i\left(\mathbf{K} \cdot \mathbf{r} - \delta\omega t\right)\right] + \text{c.c.}\} \quad (7)$$

where $I_o = |\mathcal{E}_1(z,t)|^2 + |\mathcal{E}_2(z,t)|^2$, $\mathbf{K} = \mathbf{k}_2 - \mathbf{k}_1$ and $\delta\omega = \omega_2 - \omega_1$. The sencond term corresponds to a moving interference pattern whose wavevector is **K** and whose velocity is given by dw/*K*. For simplicity, light beams which interfere symmetrically in the crystal is considered. In this case the interference formed is the direction of the x axis as shown in figure 1. That is, $\mathbf{K}\cdot\mathbf{r}$ = Kx.

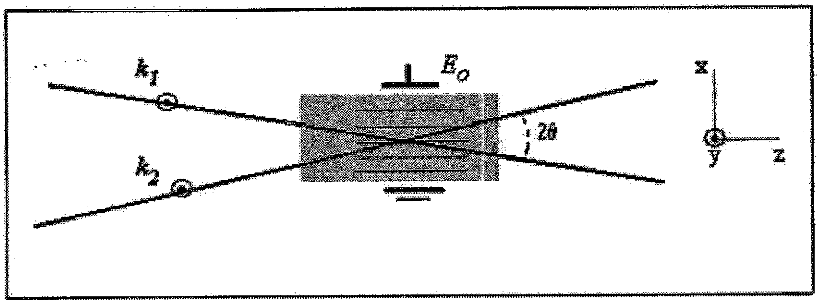

Figure 1. Two wave mixing in a drift-type crystal. The beams re polarized perpendicular to the paper, along the y-axis. An external field is applied along the x-axis.

For a wide range of applications, the following approximations can be made: 1) $n \ll N_D^+$; 2) $\beta_e \ll sI$; 3) $N_D^+ \ll N_D$; 4) $\nabla \approx \frac{\partial}{\partial x}\hat{i}$; and 5) the permittivity is independent of x. The static electric field (including the externally applied electric field) is along the x direction which dictates the direction of current density. With these approximations, equations (1-4) reduce to:

$$e\frac{\partial N_D^+}{\partial t} = \frac{\partial J}{\partial x} \tag{8}$$

$$\frac{\partial N_D^+}{\partial t} = sIN_D - \gamma_R n N_D^+ \tag{9}$$

$$J = e\mu n E_s - k_B T\mu\frac{\partial n}{\partial x} \tag{10}$$

$$\frac{\partial E_s}{\partial x} = \frac{e}{\varepsilon_o\varepsilon_s}\left(N_A - N_D^+\right) \tag{11}$$

The above equations can be combined to give:

$$sIN_D + \left(\frac{\gamma_R\varepsilon_o\varepsilon_s}{e} - \mu\right)n\frac{\partial E_s}{\partial x} - \mu\frac{\partial n}{\partial x}E_s = \gamma_R n N_A - \frac{k_B T\mu}{e}\frac{\partial^2 n}{\partial x^2} \tag{12}$$

The remaining dynamical variables in equation (12) are Es and n. Considering only up to first order spatial harmonics, they can be expressed as:

$$n(x,z,t) = n_o + \left\{\frac{1}{2}n_1(z,t)\exp\left[i\left(Kx - \delta\omega t\right)\right] + c.c\right\} \tag{13}$$

$$E_s(x,z,t) = E_o + \left\{\frac{1}{2}E_{s1}(z,t)\exp\left[i\left(Kx - \delta\omega t\right)\right] + c.c\right\} \tag{14}$$

where E_0 is the externally applied electric field and n_1 and E_{s1} are the time-dependent amplitudes of the electron density and the static field, respectively. The higher-order spatial harmonics have been neglected. Substitution of equations (13), (14) and (7) into equation (12) gives:

$$\frac{\partial E_{s1}}{\partial t} = i\delta\omega E_{s1} - \frac{E_M}{\tau_D E_q}\frac{E_o - i\left(E_q + E_T\right)}{E_o - i\left(E_M + E_T\right)}E_{s1}$$

$$+\frac{2E_M}{\tau_D}\frac{E_T + iE_o}{E_o - i\left(E_M + E_T\right)}\frac{\mathcal{E}_1^*(z,t)\mathcal{E}_2(z,t)}{I_o} \qquad (15)$$

where $E_T = (1/e)KTk_B$, $E_M = \frac{\gamma_R N_A}{\mu K}$, $E_q = \frac{eN_A}{\varepsilon_o \varepsilon_s K}$, and $\tau_D = \frac{\varepsilon_o \varepsilon_s}{e\mu n_o}$. E_T and EM are referred to as diffusion field and drift, field, respectively. E_q, the saturation field, is the maximum electric field achievable for a given N_A. T_D is the dielectric relaxation time. Since n_o can be shown to be equal to $\frac{sIN_D}{\gamma_R N_A}$, T_D can be written as:

$$\tau_D = \frac{\varepsilon_o \varepsilon_s}{e\mu}\frac{\gamma_R N_A}{sI_o N_D} \qquad (16)$$

The change in the index of refraction due to the static electric field, E_s, via the linear electro-optic effect is:

$$\Delta n = \frac{1}{2}n_b^3 r_{eff} E_s \qquad (17)$$

where r_{eff} is the linear electro-optic coefficient and n_b is the background index of refraction, respectively of the crystal. Using equation (14), equation (17) becomes:

$$\Delta n = \Delta n_o + \left\{\frac{1}{2}\Delta n_{s1}\exp\left[i\left(Kx - \delta\omega t\right)\right] + c.c.\right\} \qquad (18)$$

where

$$\Delta n_o = \frac{1}{2}n_b^3 r_{eff} E_o \qquad (19)$$

and

$$\Delta n_{s1} = \frac{1}{2}n_b^3 r_{eff} E_{s1} \qquad (20)$$

Thus from the equation (15), the amplitude, Δn_{s1}, of the index modulation is given by the following equation:

$$\frac{\partial \Delta n_{s1}}{\partial t} = C_1 \Delta n_{s1} - C_2 \Delta n_{s1} + C_3 \frac{1}{2} n_b^3 r_{eff} \frac{\mathcal{E}_1^*(z,t)\mathcal{E}_2(z,t)}{I_o} \quad (21)$$

where

$$C_1 = i\delta\omega \quad (22)$$

$$C_2 = \frac{E_M}{\tau_D E_q} \cdot \frac{E_o - i(E_q + E_T)}{E_o - i(E_M + E_T)} \quad (23)$$

$$C_3 = \frac{2E_M}{\tau_D} \cdot \frac{E_T + iE_o}{E_o - i(E_M + E_T)} \quad (24)$$

Equation (21) is the main result of this section.

B. Equation for Gain

The interaction of light with the medium is governed by Maxwell's wave equation:

$$\nabla^2 \mathbf{E}(\mathbf{r},t) - \frac{1}{c^2}\frac{\partial^2 \mathbf{E}(\mathbf{r},t)}{\partial t^2} = \mu \frac{\partial^2 \mathbf{P}(\mathbf{r},t)}{\partial t^2} \quad (25)$$

where **E** and **P** are the electric field and polarization, respectively. *c* is the speed of light in vacuum and μ is the permittivity. P has both linear and nonlinear components with the linear polarization given by:

$$\mathbf{P}_L(\mathbf{r},t) = \varepsilon_o \chi_L \mathbf{E}(\mathbf{r},t) = \varepsilon_o \left(n_b^2 - 1\right) \mathbf{E}(\mathbf{r},t) \quad (26)$$

where $_{XL}$ is the linear dielectric susceptibility of the crystal. In the presence of two coherent light beams in the crystal, the linear polarization given by equation (26) can be generalized to give the following equation for polarization:

$$\mathbf{P}(\mathbf{r},t) = \varepsilon_o \chi \mathbf{E}(\mathbf{r},t) = \varepsilon_o \left[(n_b + \Delta n(\mathbf{r},t))^2 - 1\right] \mathbf{E}(\mathbf{r},t) \quad (27)$$

where $\mathbf{E} = \mathbf{E}_1(\mathbf{r},t) + \mathbf{E}_2(\mathbf{r},t)$. $\Delta n(\mathbf{r},t)$. $\Delta n(\mathbf{r},t)$ is the induced change in the index of refraction via linear-electro-optic effect due to static electric field and is given by equation (17). Substitution of equation (27) into equation (25) and considering assumptions discussed in the previous subsection, the result is:

$$\frac{\partial^2 E(z,t)}{\partial z^2} - \frac{1}{v^2}\frac{\partial^2 E(z,t)}{\partial t^2} = -\frac{2}{n_b}\frac{\Delta n}{v^2}\frac{\partial^2 E(z,t)}{\partial t^2} \quad (28)$$

where $v = \frac{c}{n_b}$ is the velocity of light in the crystal.

Equation (28) can be transformed into equations involving the complex amplitudes by assuming that the complex amplitudes of the fields vary very slowly in z and t compared to the wavenumbers and optical frequencies. This is called the slowly varying amplitude approximation and mathematically means that $k_i^2 \gg \frac{\partial^2 \mathcal{E}_i}{\partial z^2}$ and $\omega \gg \frac{\partial^2 \mathcal{E}_i}{\partial t^2}$ (where i=1,2). The interest here is in the steady-state gain properties of the crystal and therefore we set $\frac{\partial \mathcal{E}_1}{\partial t} = \frac{\partial \mathcal{E}_2}{\partial t} = \frac{\partial \Delta n_{s1}}{\partial t} = 0$. Substitution of equations (5), (6), (7) and (17) into equation (28) gives:

$$\frac{\partial \mathcal{E}_1(z)}{\partial z} = \frac{i2\pi}{\lambda \cos\theta} + \frac{\Delta n_o}{n_b}\mathcal{E}_1(z) + \frac{i\pi}{\lambda \cos\theta}\frac{\Delta n_{s1}^*}{n_b}\mathcal{E}_2(z) \quad (29)$$

$$\frac{\partial \mathcal{E}_2(z)}{\partial z} = \frac{i2\pi}{\lambda \cos\theta} + \frac{\Delta n_o}{n_b}\mathcal{E}_2(z) + \frac{i\pi}{\lambda \cos\theta}\frac{\Delta n_{s1}}{n_b}\mathcal{E}_1(z) \quad (30)$$

where the coherent light beams are assumed to have the same wavelength, that is $k_1 = k_2 = k = \frac{2\pi}{\lambda}$. The grating wavenumber is $K=2k \sin \theta$, where 2θ is the angle between the beams as shown in figure 1. Thus the grating wavelength is given by:

$$\Lambda = \frac{2\pi}{K} = \frac{\lambda}{2\sin\theta} \quad (31)$$

From equation (21), the refractive index modulation at steady-state is:

$$\Delta n_{s1} = \frac{\frac{1}{2} n_b^3 r_{eff} C_3}{C_2 - C_1} \frac{\mathcal{E}_1^*(z)\mathcal{E}_2(z)}{I_o} \quad (32)$$

Δn_{s1} is complex and can be expressed in terms of real amplitude, Δn_1 and phase, φ:

$$\Delta n_{s1}(z) = \Delta n_1 e^{i\varphi} \quad (33)$$

The electric field amplitudes can likewise be written in terms of intensity and phase as:

$$\mathcal{E}_1(z) = \sqrt{I_1(z)} \exp(i\psi_1(z)) \quad (34)$$

$$\mathcal{E}_2(z) = \sqrt{I_2(z)} \exp(i\psi_2(z)) \quad (35)$$

Using equations (33)-(35), equations (29) and (30) become:

$$\frac{dI_1(z)}{dz} = \gamma\frac{I_1(z)\,I_2(z)}{I_o} - \alpha I_1(z) \qquad (36)$$

$$\frac{dI_2(z)}{dz} = -\gamma\frac{I_1(z)\,I_2(z)}{I_o} - \alpha I_2(z) \qquad (37)$$

$$\frac{d\psi_1(z)}{dz} = \beta_o + \beta_1\frac{I_2(z)}{I_o} \qquad (38)$$

$$\frac{d\psi_2(z)}{dz} = \beta_o + \beta_1\frac{I_1(z)}{I_o} \qquad (39)$$

where

$$\gamma = \frac{2\pi}{\lambda n_b\cos\theta}\Delta n_1\sin\varphi \qquad (40)$$

$$\beta_1 = \frac{\pi}{\lambda n_b\cos\theta}\Delta n_1\cos\varphi \qquad (41)$$

$$\beta_o = \frac{2\pi}{\lambda n_b\cos\theta}\Delta n_o \qquad (42)$$

The second terms in equations (36) and (37) have been added to account for the losses due to the absorption of light by the crystal. The solution to these equations are well known [9] and this is given by the following equations:

$$I_1(z) = I_1(0)\frac{1+m}{1+me^{-\gamma z}}e^{-\alpha z} \qquad (43)$$

$$I_2(z) = I_2(0)\frac{1+m^{-1}}{1+m^{-1}e^{-\gamma z}}e^{-\alpha z} \qquad (44)$$

where $m=I_2(0)/I_1(0)$. The solution reflects the transfer of energy from one signal into the other as shown in figure 2 for $m=1$. The result can be interpreted in terms of self-diffraction. Each output signal consists of the original beam and the diffracted beam of the other. One of the output light corresponds to constructive interference (increased intensity) and the other to destructive interference (decreased intensity). If m >> 1, and $\gamma > \alpha$, the weak signal is amplified and $I_1(z) = I(0)\exp[(\gamma-\alpha)z]$ while $I_2(z) >> I_2(0)$. In this case, $I_2(0)$ and $I_1(z)$ are referred to as the pump intensity and signal intensity, respectively.

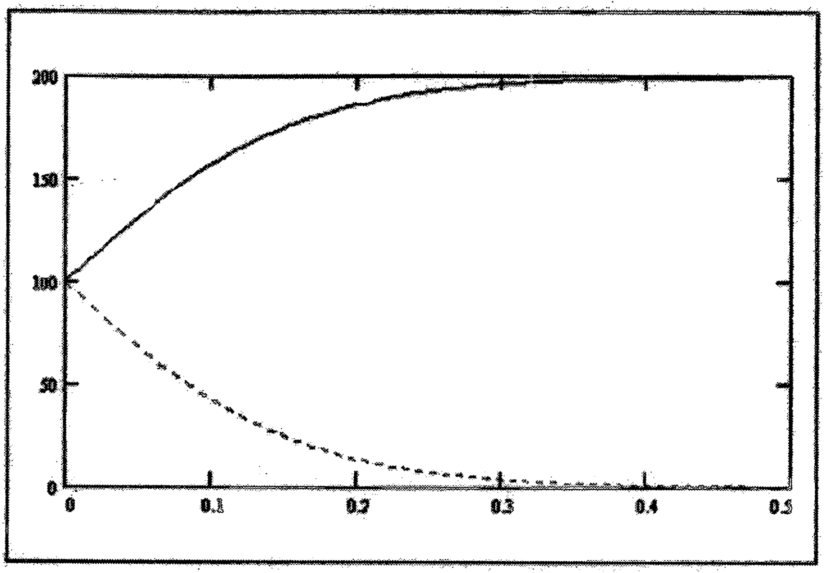

Figure 2. Intensities of the beams as they propagate through the crystal.

Our interest is on the dependence of the gain on the controllable parameters—applied electric field, E_0, grating wavelength, Λ, total intensity, the ratio of intitial intensities, m, and the frequency shift, δω, of the signal beam relative to the strong pump beam. The latter is related to the velocity, υ, of the grating movement by the following equation:

$$\delta\omega = Kv \tag{45}$$

From equation (43), the linear gain, Γ, is by:

$$\Gamma = \frac{1+m}{1+m\exp(-\gamma z)} \tag{46}$$

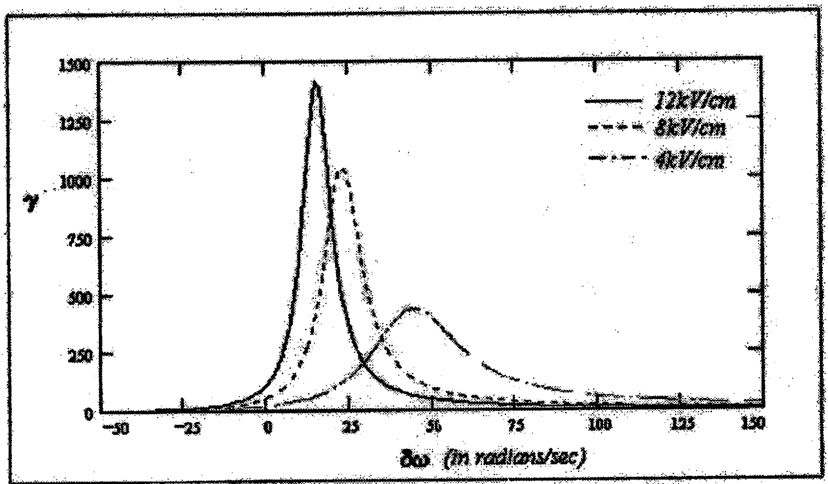

Figure 3. Gain coefficient as a function of the frequency shift for grating wavelength of 10 microns, pump intensity of 10 mW/cm² and m=1000.

and therefore the actual gain coefficient, γ', is given by:

$$\Gamma = \exp(\gamma' z) \tag{47}$$

Note that γ'' should be differentiated from the material gain, γ', which is given by equation (40).

III. Numerical Simulation

From equations (32), (33) and (40), the following equation for gain coefficient, γ, is obtained:

$$\begin{aligned} \gamma &= \frac{2\pi}{n_b \lambda \cos\theta} \operatorname{Im}\left(\Delta n_1 e^{i\varphi}\right) \\ &= \frac{\pi n_b^2 r_{eff}}{\lambda \cos\theta} \operatorname{Im}\left(\frac{C_3}{C_2 - C_1}\right) \end{aligned} \tag{48}$$

The phase shift, φ of the grating relative to the interference pattern can likewise be obtained.

$$\varphi = \tan^{-1} \frac{\operatorname{Im}\left(\frac{C_3}{C_2 - C_1}\right)}{\operatorname{Re}\left(\frac{C_3}{C_2 - C_1}\right)} \tag{49}$$

C_1, C_2 and C_3 are complex and involve not only the external field, E_o, but also E_q, E_T, E_M and T_D. The last four quantities in turn depend on intrinsic crystal properties and external parameters I_0 and λ and Λ.

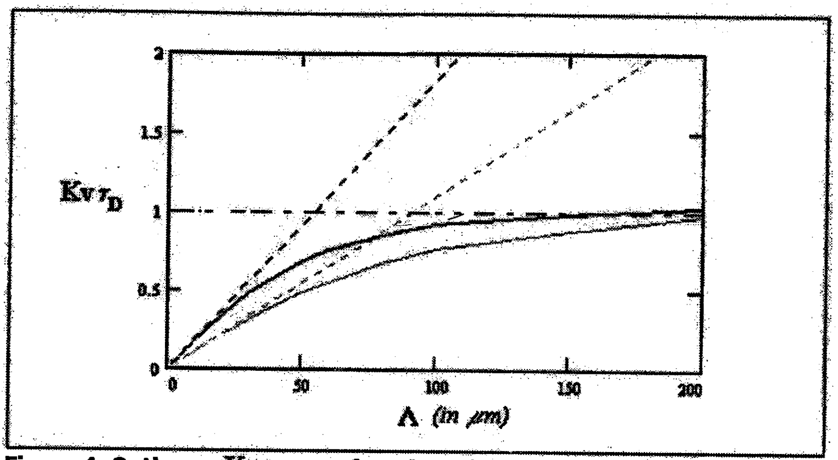

Figure 4. Optimum $Kv\tau_D$ as a function of grating wavelength. The numerical results (solid lines) are compared with those obtained using Huignard's approximation (dadot) and Refrieger (dotted lines). The light and dark solid lines corresponds to E_0 equal to 10 and 6 kV/cm², respectively.

From equations (46) — (48), the actual linear gain, Γ, and gain coefficient, γ', can be obtained. If the interest is simply on the general gain properties of the crystal in conventional two-wave mixing configuration, these results hold for both diffusion-type and drift-type crystals if the assumptions already discussed are satisfied. In general, as in some diffusion-type crystals (e.g. barium titanate), the gain strongly depends on the direction of the interference relative to the c-axis of the crystal. For many drift-type crystals, the configuration is as shown in figure 1 with vertically-polarized input beams.

For drift-type crystals, equations (48) and (49) reduce to the results of Huignard et. al [7] for the case of $E_o > E_M$, E_q and to that of Refrieger et. al [8] for the case of negligible Et and $E_o > E_M$. In the following, equation (48) is solved numerically with no further approximations other than those already discussed in section II. The choice of direct numerical solutions rather that approximate analytical solution is dictated by the crystal properties, particularly that of BSO whose typical crystal properties are given in Table 1. From this, the following values are obtained (in units of kV/m): $E_\tau = 165/\Lambda$, $E_M = 11\Lambda$ and $E_q = 510L$. For $\Lambda = 10\mu m$, Et, E_M and E_q are equal to 16.5, 110 and 5100 kV/m, respectively. For a typical value of $E_o = 1000$ kV/m (=10kV/m), Huignard approximation does not hold. In reference to the Refrieger approximation, E_τ is not negligibly small compared to E_M.

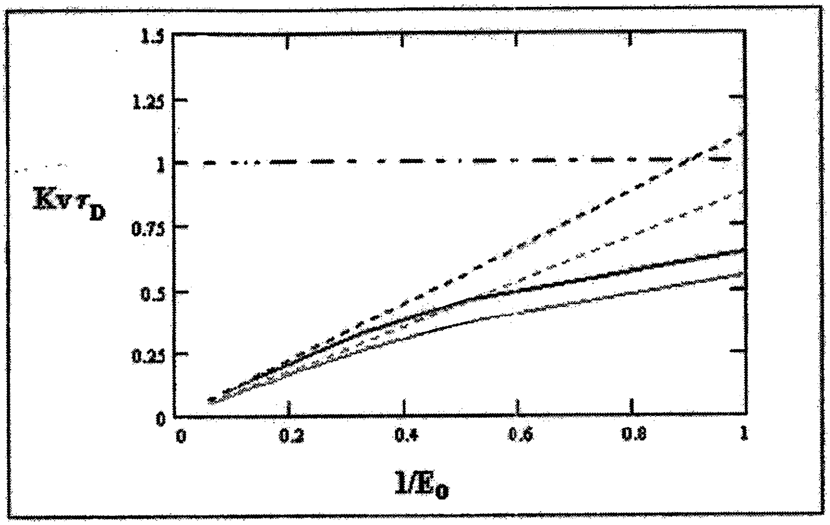

Figure 5. Optimum $Kv\tau_D$ as a function of the inverse of the external field. Numerical results (solid lines) are compared with those using Huignard (dadot) and Refrieger (dotted lines) approximations. The dark and light solid lines correspond to the grating wavelength equal to 10 and 8 microns, respectively.

In numeral solutions which follow, the electric fields are normalized to E_o to give the following expression obtained from equations (22), (23) and (24):

$$\frac{C_3}{C_1 - C_2} = \frac{2E_q\left(\hat{E}_T + i\right)\left[-i\left(\delta\omega T + \hat{E}_q + \hat{E}_T\right) + \delta\omega T\left(\hat{E}_M + \hat{E}_T\right) - 1\right]}{\left[\delta\omega T + \hat{E}_q + \hat{E}_T\right]^2 + \left[\delta\omega T\left(\hat{E}_M + \hat{E}_T\right) - 1\right]^2} \qquad (50)$$

where $T = \frac{\tau_D E_q}{E_M}$. Using values in Table 1 and with E_o in units of kV/cm, the normalized values of the fields are: $\hat{E}_T = \frac{1.65}{\Lambda E_o}$, $\hat{E}_M = \frac{0.11\Lambda}{E_o}$ and $\hat{E}_q = \frac{5.1\Lambda}{E_o}$.

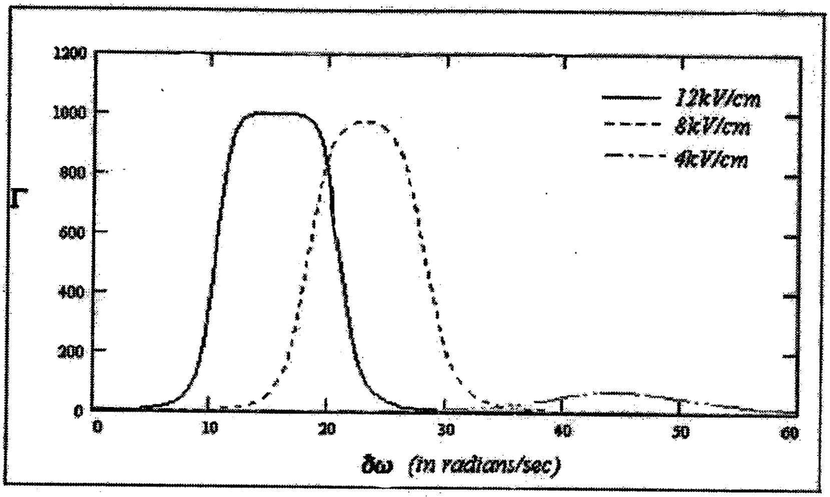

Figure 6. Actual gain as a function of the frequency shift for grating wavelength, pump intensity and intensity ratio equal to 10 micron, 100 mW/cm2 and 2000, respectively, for the three different values of external field.

The gain coefficient, γ (obtained from eqns. (48) and (50)) is plotted as a function of $\delta\omega = K\upsilon$ in figure 3. Unlike the diffusion-type crystals, the plot suggests that optimum gain depends on E_o and corresponds to a nonstationary grating whose velocity also depends on E_o.

The numeral results suggests the nonlinear dependence of $Ku_{op}t_D$ on grating wavelength, Λ and $1/E_o$ as shown in figures 4 and 5. Earlier results [7] based on the approximation: $E_o > E_M, E_q$ gives the commonly quoted result for the optimum velocity given by: $K\upsilon_{op}\tau_D \approx -1$. The less restrictive approximation [8], which is referred to here as the Refrieger approximation, on the other hand shows a linear dependence of the function, $K\upsilon_{op}\tau_D$, on $1/E_o$ and Λ, also shown. For large E_o and small Λ, this latter approximation agrees with the numerical results. Otherwise, the deviations of the results based on this approximation from that of the numerical calculations are evident.

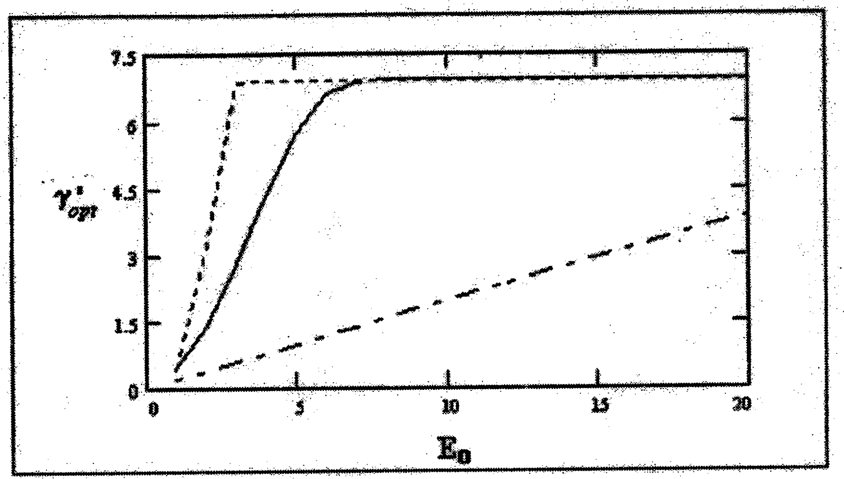

Figure 7. Optimum actual gain as function of external field for grating wavelength, pump intensity and intensity ratio equal to 10 micron, 100 mW/cm² and 1000, respectively. The numerical results (solid line) are compared with those using Huignard (dadot) and Refrieger (dotted line) approximations.

In Huignard's approximation, τ_D (which depends on I_0) can be interpreted as the characteristic time for grating formation. The condition, $K\upsilon_{op}\tau_D \approx -1$, simply expresses that the optimum gain is attained when the grating does not move too fast or too slow compared with the formation of grating itself. This condition is not generally correct as shown in figures 4 and 5, but a time τ' can be defined such that $K\upsilon_{opt}, \tau_D \approx -1$. In this case τ' is just the inverse of the optimum frequency shift, $\delta\omega_{opt} = K\upsilon_{opt}$. Thus in general, for drift-type crystal, the characteristic time for grating formation is τ' which is now a function not only of I_0 but also of Λ and E_0.

Because of the dependence of the actual gain coefficient, γ' given by eqns. (46) and (47) on the ratio of the intensities of the beams, it shows saturation for large external field as shown in figure 6. This is more evident in figure 7 where the optimum actual gain γ'_{opt}, is plotted as functions of E_0.

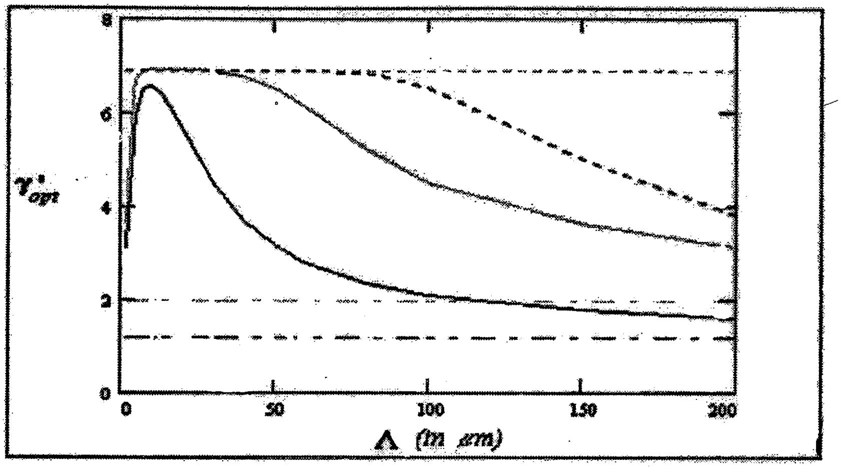

Figure 8. Optimum actual gain as a function of the grating wavelength for pump intensity and intensity ratio equal to 100 mW/cm^2 and 1000, respectively. The numerical results (solid lines) are compared on those based on Huignard (dadot lines) and Refrieger (dotted lines) approximation. The dark and light solid lines correspond to external field equal to 10 and 6 kV/cm, respectively.

In these results the BSO crystal is assumed to have length equal to 1 cm. For comparison, the results using Huignard and Refrieger's are also plotted. Both the numerical result and that based on the Refrieger approximation show saturation of optimum gain for some value of E_0, although saturation occurs earlier in the latter. In figure 8, the dependence of γ'_{opt} on Λ for two values of E_0 are shown. Both the results from the Refrieger approximation and the numerical results, show the existence of a range of Λ where γ' is maximum. This range depends on the external field and is absent for small values of E_0. Both results agree for small values of Λ, the results based on Refrieger's approximation, however, show very large range of Λ corresponding to maximum actual gain.

This section presented the results based on the direct numerical calculation of Kukhtarev's equations for the BSO crystal. The differences between these results and that based on two approximations which are commonly quoted in the literature are highlighted. In the following section, experimental results are presented.

Table 1. Material properties of Bismuth Silicon Oxide (BSO).

dielectric constant, ε_s	56
refractive index, η_b	2.54
recombination rate, γ_R	2 X 10^{-17} m3/sec
acceptor density, N_A	$10^{22}/m^3$
mobility, μ	0^{-5} m^2/V-sec
electro-optic coefficient, r_{eff}	5 x 10^{-12} m/V
cross-section of photoionization, s	1.06 x 10^{-5} m^2/J
donor density, N_D	$10^{25}/m^3$

IV. Experiments

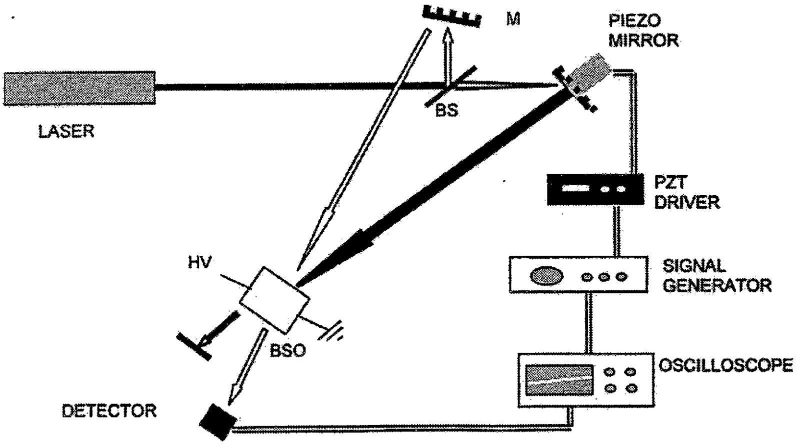

Figure 9. Schematic diagram of the experimental set-up.

The experimental set-up is shown in figure 9. The coherent light source is a vertically polarized beam (with wavelength equal to .514µm) from an air-cooled argon-ion laser. The argon-ion laser used is only one-fourth meter long. With a linewidth of 3 Ghz, the argon laser is expected to oscillate with at least two longitudinal modes when it is operated at its maximum power of 130 mW. However, in most of the experiments performed, the laser was operated well below this peak power and it is reasonable to assume that the laser beam has only one oscillation frequency.

The beam is split into two by a beam splitter one of thich is the pump beam that goes directly to 5x5x10 mm BSO crsytal and the other is the signal beam goes first to a mirror which reflects it to the crystal. The wavelength of the grating formed by the pump and signal beams is determined by the angle between these beams while the ratio of the intensities of the beams before the crystal is controlled by neutral density filters in the path of the signal beam. The grating formed in the crystal is moved by linearly scanning the mirror using a PZT crystal driven by a power supply. Since BSO is a drift-type crystal, an external field is applied perpendicular to the optical axis of the crystal.

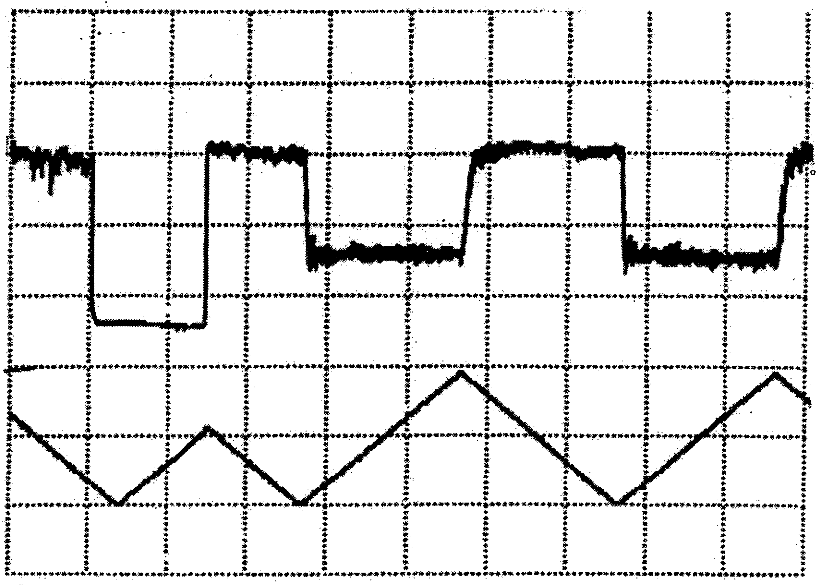

Figure 10. Oscilloscope display of weak signal amplification. The signal is amplified only in the correct direction of mirror motion.

The signal beam is detected by a photodiode which in turn produces electrical signal which is viewed on an oscilloscope. Since the oscilloscope display is proportional to the signal beam intensity, the ratio of the intensity of the signal beam in the presence of the pump beam over the signal beam intensity without the pump beam can be determined. It can be shown from equation (43) that this ratio is simply the linear gain, Γ. A typical display on the oscilloscope is shown in fig.10. The voltage scan on the mirror (also shown) determines the direction and magnitude of the mirror velocity (which is directly proportional to the grating velocity). Since the optimum gain is sensitive to the grating velocity, amplification occurs in only one direction of the grating motion.

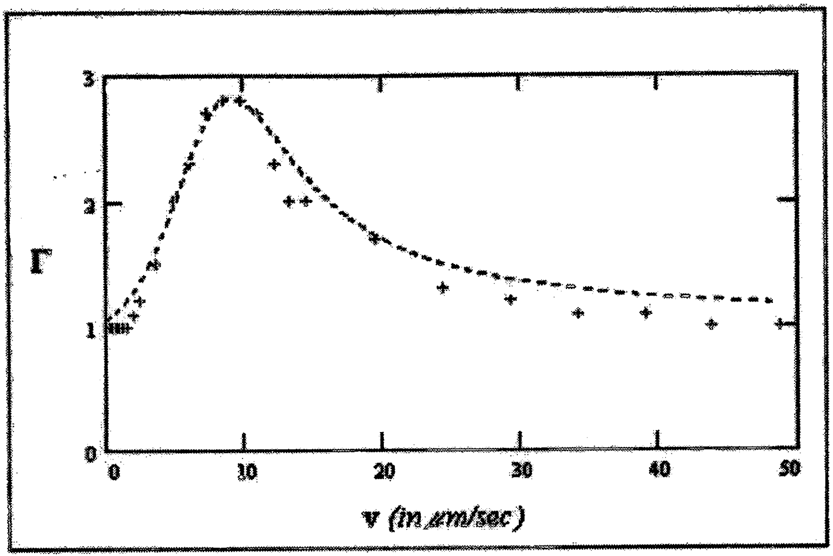

Figure 11. Linear gain as a function of the grating velocity for E_0 and grating wavelength equal to 5 kV/cm and 25.8 micron, respectively. The theoretical fit is also shown (dotted line).

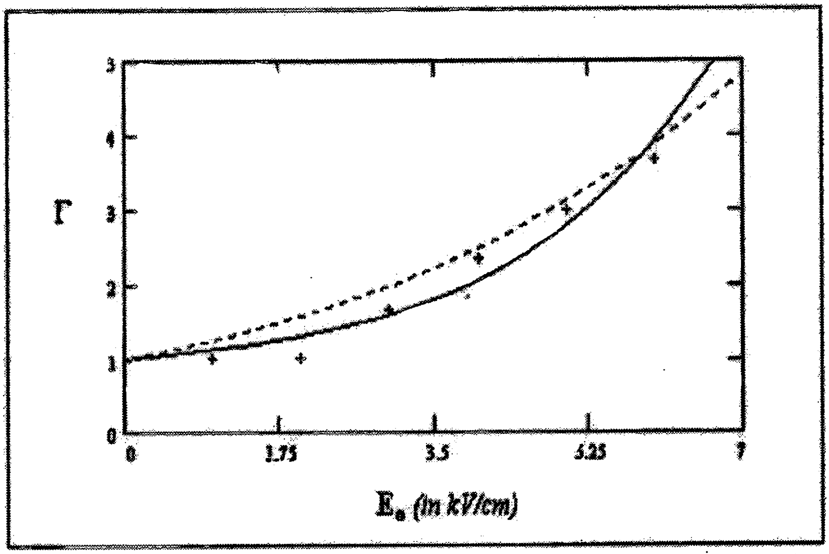

Figure 12. Linear gain as a function of external field for grating velocity and grating wavelength equal to 25 micron/sec and 29.4 micron, respectively. The theoretical fit (solid line) based on the model discussed is shown and compared with the theoretical fit based on Huignard's approximation (dotted line).

The linear gain is plotted as a function of grating velocity for $E_o = 5$kV/cm, $m = 291$ and $\Lambda = 25.8\mu m$ in figure 11. As predicted, there is an optimum grating velocity corresponding to a set of E_o and Λ. The linear gain as a function E_o for $\upsilon = 25\mu m/$ sec, m = 1214 and $\Lambda = 29.4$, μm is plotted in figure 12. The nonlinear increase in linear gain is consistent with the theoretical predictions. To see this, the theoretical fit using the numerical calculations discussed in this article is compared with the fit obtained using Huignard's approximation.

Since crystal are not expected to have exactly the same values for most of its material properties (such as N_A, N_D, γ_R and μ), the theoretical curves(drawn in figures 11-12) have been generated to fit the experimental results. This in turn allows the determination of ratios of values of the material properties. From these, the values of N_A and γ_D/μ for the crystals used are .95 x 10^{22} /m^3 and 1.65 x 10^{-12} m/V, respectively. These are of the same order of magnitude as the values quoted in the literature (see table 1).

V. Conclusion

From Khuktarev's equations, a numerical model for two-wave mixing in a photorefractive crystal has been formulated and expressed in terms of an equation for modulation amplitude of the index of refraction induced in the crystal. This form of the material equation is a convenient starting point for a theoretical model addressing the dynamical behaviour of an oscillator with a drift-type crystal as an amplifying medium.

The numerical model discussed in this paper has been applied to a drift-type crystal to study the steady state amplification of a coherent weak signal. The actual gain depends on the external field applied to the crystal, the grating velocity, the grating wavelength and the ratio of beam intensities. The maximum gain occurs at a specific grating velocity, the magnitude of which depends on the external electric field, grating wavelength and pump intensity. While most of these results have been reported, the dependences of the optimum gain and grating velocity on the parameters as predicted by earlier studies deviate from the results obtained by the numerical model developed in this paper. Inspite of the experimental conditions which did not allow the optimum amplification of the weak signal, the experimental results nevertheless, are consistent with the theoretical results.

ACKNOWLEDGEMENTS

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Insect Floral Visitors of Four Reforestation Tree Species in Mt. Maliking, Luzon Philippines[1]

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ABSTRACT

Observations were conducted on the insect pollinators of four reforestation tree species in Mt. Makiling, Laguna Province, Southern Luzon, Philippines namely: Acacia auriculiformis *Cunn.* ex *Benth. (auri),* Vitex parviflora *Juss. (molave),* Leucaena leucocephala *(Lam.) de Wit (ipil-ipil, El Salvador Strain) and* Pterocarpus indicus *Willd.*[3] *(narra). Washings were made from captured specimens of insect floral visitors of each reforestation species and pollen grains were extracted and counted. Importance of floral visitors was based on the values of the percentage pollen extracted on the total pollen count including the total number of insect visits per hour. Floral morphology of each of the reforestation species and insect foraging behavior were described.* A. auriculiformis *exhibits an "open" flower suggesting general mellitophily.* Megachile *sp. appears to be an important visitor based on the pollen load and number of visits per spike per hour. The flower of* P. indicus *is specialized, requiring some tripping mechanism before the nectar and pollen can be extracted. The pollinators are medium to large-sized bees, notably* Xylocopa sp. V. parviflora *is likewise a bee flower, being visited by several species of medium to large-sized bees.* L. leucocephala *is also visited by* Xylocopa sp. *and species of* Vespidae.

INTRODUCTION

Forest trees are pollinated by a wide variety of animals such as bats, rodents, lemurs, birds and insects. Among the insects, the bees are the most important group of pollinators based on the number and diversity of plant species visited and pollinated. Trees commonly pollinated by bees belong to the families Burseraceae, Euphorbiaceae, Flacourtiaceae, Lecythidaceae and Sapotaceae (Bawa et al., 1985; Ormond et al., 1981).

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[1] Portion of the senior author's Ph D thesis submitted to the Graduate School, University of the Philippines Los Baños, College, Laguna, March 1997.

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[3] The species has been revised (Rojo, 1972), i.e., the smooth narra is *forma indicus* and the prickly narra is *forma echinatus* (Pers.) Rojo. Smooth narra is the subject of this study.

Aside from visiting orchids, solitary bees also visit trees and other tropical plants (Janzen, 1971). The bee pollination syndrome is reported dominant in canopy trees (Bawa et al., 1985).

For planning and programming purposes, the reproductive biology and pollination ecology of recommended reforestation species for the plantation and reforestation projects in both government and private sectors should first be known prior to mass utilization of these species. This is to ensure adequate quantity and high quality of fruits and seeds produced by naturally outcrossing reforestation species. Such natural regeneration greatly depends on the activities and availability of pollinators in the area (Oni, 1991). Therefore, in order to establish plantation of trees with superior genotypes and high growth vigor, adequate knowledge on the pollination ecology of the desired species is needed.

Very little is known about the reproductive biology and pollination ecology of the reforestation and plantation species utilized at present. Scarcity of basic information on this aspect may be associated with the physical and technical difficulties of conducting pollination studies on forest trees. Unlike agricultural crops, trees attain considerable height upon maturity and usually produce short-lived flowers. The height and the irregularity in the production of flowers pose problems in the observation of insect pollinators and determination of breeding systems of forest trees. Present knowledge on pollination ecology of forest trees is solely based on studies conducted abroad, and this state of affairs should not be allowed to continue.

As part of a series, the present study was conducted to identify floral visitors and pollinators of four reforestation species in Mt. Makiling, Laguna Province, Southern Luzon, Philippines.

MATERIALS AND METHODS

Selection and Preparation of Reforestation Tree Species

Exotic or introduced (*A. auriculiformis* and *L. leucocephala*) and native species (*V. parviflora* and *P. indicus*) were represented in the selection process. Three to four trees per species were selected at random, marked with white paint and tagged for easy recognition. Prior to actual field observation and data gathering permanent bamboo ladders and scaffoldings were built for each tree for human access to the tree tops and branches (Figure 1).

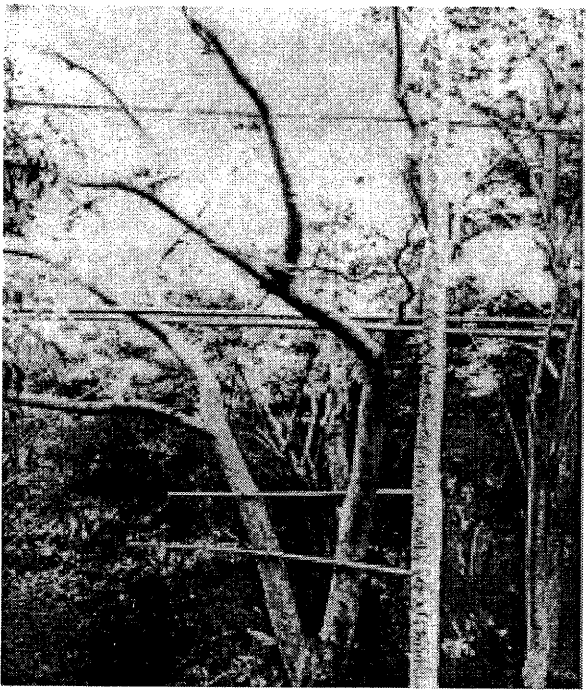

Figure 1. Bamboo ladders and scaffoldings installed prior to actual field observation and data gathering to provide access to the flowers of *P. indicus.*

Figure 2. Plantation of L. leucocephala in Mt. Makiling.

Randomly selected trees measured at least 10 cm diameter at breast height and 10 meters high. They were carefully examined for diseases and herbivore damage that could significantly affect flowering and fruiting performance.

Floral Visitors

With researchers perched on tree tops and branches, floral visitors were observed as they landed on the flowers. Observations were made from 0700H to 1700H. For the first 15 minutes of each hour, the visitors and the frequency of each visit were noted. Population density of each floral visitor was based on the number of visits per inflorescence per hour.

Specimens of floral visitors were captured and sent to the University of the Philippines Los Banos Museum of Natural History (UPLB-MNH) for identification.

Pollen Load of Floral Visitors

Some floral visitors of each tree examined were caught and preserved in bottles with ethyl alcohol-soaked cotton. For each visitor, at least one was selected and washed separately with distilled water and alcohol in separate petri dishes. Each washing was thoroughly stirred in a beaker with a glass rod and from it, five slides were prepared by adding a drop of washing (about 0.1 cu cm) to each slide. In each slide, pollen grains were counted in five fields of view along the length of a diagonal, and the mean was obtained. Thus, 25 observations were made for each floral visitor caught in each tree species. The total number of pollen in the original washing was computed and expressed for each visitor as a percentage of the total number for all species. Floral visitor importance was based on the amount of pollen load extracted from the washing.

RESULTS

A. *A. auriculiformis*

A total of nine insects were observed foraging in the flowers of *A. auriculiformis*. They were generally identified as *Coccinelidae, Simuliidae, Lygaedae, Chrysomelidae, Anthoporidae, Tabaniidae, Syrphidae, Pyrrhococcidae* and *Megachiliidae:Megachile* sp. In terms of average visit per spike per hour, *Megachile* sp. showed the highest value at 6.86 (Table 1). The rest showed about 2 visits. The insect visitors were active from 0700H to about 1400H. As shown in Table 2, *Megachile* sp. had about 99.43% of all pollen counted. Other visitors carried about 0.5%, some with pollen belonging to another plant or without any pollen load.

Table 1. Average visit of floral visitors per spike in *A. auriculiformis.*

Floral visitors	Total number of visits from 0700 – 1800H	Average visit per spike/hour
Coccinelidae	20	2.86
Simuliidae	16	2.28
Lygaeidae	20	2.86
Chrysomelidae	16	2.28
Anthoporidae	12	1.71
Tabaniidae	12	1.71
Syrphidae	12	1.71
Pyrrhococcidae	20	2.86
Megachiliidae : Megachile sp.	48	6.86

Table 2. Floral visitors and pollen load in *A. auriculiformis.*

Floral visitors	Pollen carriage/ load	% of total pollen counted
Syrphidae (1)*	0	0
Syrphidae (2)*	0	0
Pyrrhococcidae-Dysdercus sp.	60	0.5
Megachiliidae : Megachile sp.**	10,480	99.5
Tabaniidae	0	0
*Anthoporidae**	0	0
Chrysomelidae	0	0
Lygaeidae-Lygus sp.	0	0
Simuliidae	0	0
Total pollen counted	10,540	100

*Pollen load analyzed was from another plant.
**Visitor had a pure load of *A. auriculiformis* pollen.

Megachile sp. probed almost all the flowers in a spike for pollen grains, each probe lasting about 5 to 10 seconds. The insect landed on the spike and crawled over the entire length, continuously scraping the anthers with its pair of forelegs and raking with the hindlegs. Vibrations on the thoracic region of the insect implied that the polyads were harvested using vibration as described in *Bombus* by Heinrich (1976). Other visitors landed on the spike but no clear floral probing was observed. Their behavior seemed more of a reconnaissance survey or familiarization with the flowers. The Chhrysomelidaean visitor moved slowly and at times was immobile, eating floral parts such as stamens.

B. *V. parviflora*

There were 10 families of insects observed foraging on the flowers of *V. parviflora* namely; *Xylocopidae*: *Xylocopa* sp., *Apidae*: *Apis* sp., *Anthoporidae, Colletidae, Syrpidae, Pieridae, Caliphoridae, Halictidae, Colletidae: Euryglossa* sp. and *Chrysomelidae :Paraluperoidesp.*. The total pollen extracted and counted from the visitors was 21,700, of which 33.8% came from *Xylocopa* sp., 28% from *Apis* sp.and 25% from *Anthoporidae*. The rest had 5.3 to 1.8% of the total pollen count, while *Chrysomelidae* had no pollen load (Table 3). All the floral visitors exhibited fast and random foraging behavior.

Table 3. Floral visitors and pollen load in *V. parviflora.*

Floral visitors	Pollen carriage/ load	% of total pollen counted
Xylocopidae: Xylocopa sp.	7,340	33.8
Apidae: Apis sp.	6,080	28.0
Anthoporidae	5,640	25.9
Colletidae	620	2.8
Syrpidae	520	2.8
Pieridae	0	0
Caliphoridae	0	0
Halictidae	1,160	5.3
Collectidae: Euryglossa sp.	340	1.8
Chrysomelidae	0	0
Total pollen counted	21,700	100

Xylocopa sp. hovered in selected flowers in a panicle for about 2-3(5) seconds in each flower (Fig. 3). Thoracic vibration was audible suggesting that the pollen could have been expelled from the anther and attached and/ or deposited to the thoracic region. The species was clearly foraging for nectar in the corolla tube. It inserted its mouthparts into the tube and probed for the nectar at the base of style (Fig. 4) in nototribic manner as described by Macior (1974). The ventral portion of the head and thoracic region touched the stamens and stigmatic surfaces during nectar extraction. After the nectar was extracted, the species quickly hovered at random to another flower in the same panicle or to another panicle in another tree.

Apis sp., *Halictidae* and *Anthoporidae* likewise hovered and landed on the flower and inserted their mouthparts in the corolla tube for nectar, then transferred randomly to other flowers in the same panicle or to other panicles. The *Pieridaean* visitor inserted its proboscis in the corolla tube. The flea beetle (*Chrysomelidae*) landed on the flower and went inside the

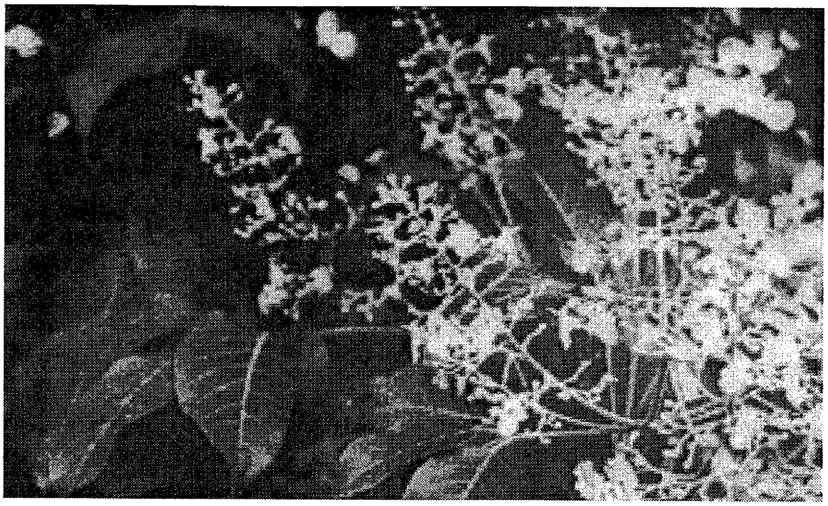

Figure 3. Infloresecene of *V. parviflora* with Xylocopa sp. foraging for nectar.

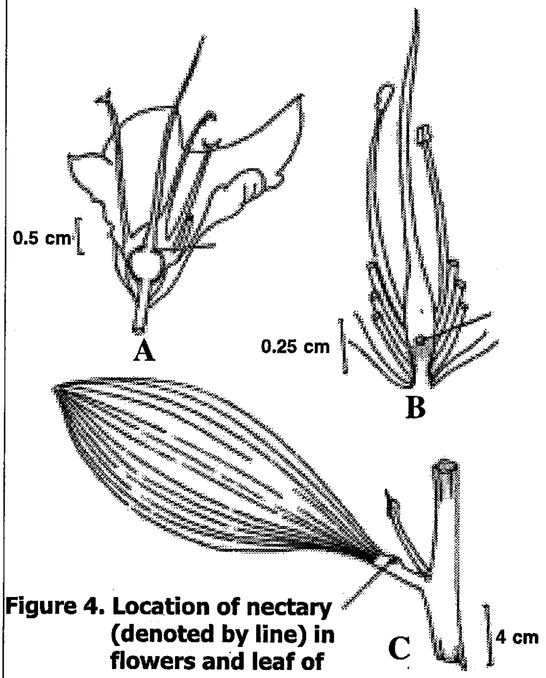

Figure 4. Location of nectary (denoted by line) in flowers and leaf of reforestation tree species studied. A. *V. parviflora*; B. *P. indicus*; C. *A. auriculiformis*.

floral tube in search for floral rewards. The visitors were active at 0700H to 1100H. The large bees were aggressive and tried to drive away the smaller bees but failed.

The average number of visit of the floral visitors per panicle per hour is presented in Table 4. The highest number of visit was made by *Xylocopa* sp. followed by *Apis* sp. and *Anthoporidae*.

Table 4. Average visit per panicle per hour in *V. parviflora*.

Floral visitors	Total number of visits from 0700–1800H	Average visit/ panicle/hour
Chrysomelidae	24	3.43
Caliphoridae	32	4.57
Pieridae	44	6.28
Syrpidae	84	12.01
Halictidae	232	33.14
Colletidae	120	17.14
Colletidae: Euryglossa sp.	140	20.00
Anthoporidae	324	46.28
Apidae: Apis sp.	328	46.28
Xylocopidae: Xylocopa sp.	372	53.14

C. *L. leucocephala*

In contrast with other species examined, only two species of floral visitors were observed foraging in the inflorescence of *L. leucocephala* within a month of observation, namely: *Xylocopa* sp. and *Vespidae*. *Xylocopa* sp. carried 26.5% of the total pollen counted. *Vespidae*, on the other hand accounted for 73.5% of the total. It should be noted that both visitors also had pollen belonging to another plant. The average visit per head per hour for *Xylocopa* sp. was 1.14, while *Vespidae* showed 0.5. Both visitors landed on the inflorescence and swifltly (about 5 to 7 seconds) crawled over the flowers. Thoracic vibrations were audible suggesting that the pollen were collected and deposited at the thoracic region. Clearly, the visitors were foraging for pollen. Afterwards, visitors swiftly transferred to another floral head. Floral visitors were very few as reflected in the average number of visits per floral head per hour.

D. *P. indicus*

Nine species of floral visitors were observed foraging in the inflorescence of *P. indicus*. *Nomia* sp., *Syrphidae*-2 and *Cicadellidae* had no pollen load, while *Vespidae*-2 and *Syrphidae* had pollen loads from other plant aside from pollen of *P. indicus*. *Xylocopa* sp. had a pure load of *P. indicus* pollen, accounting for 77.1% of all pollen counted (Table 5).

Table 5. Floral visitors and analysis of pollen load in *P. indicus..*

Floral visitors	Pollen carriage/ load	% of total pollen counted
Vespidae-1	600	5.1
Vespidae-2	962	8.2
Apidae: Apis cerana Fab.	238	2.0
Tachinidae	348	3.3
Halictidae: Nomia sp.	0	0
Apidae: Apis dorsata	504	4.3
Syrpidae	0	0
*Cicadellidae**	0	0
Xylocopidae: Xylocopa sp.**	9,003	77.1
Total pollen counted	11,691	100

*These visitors had pollen belonging to different plants.
**Visitor had only P. indicus pollen load.

Visitors hovered and landed on the inflorescence through the wing and keel petals and foraged for floral rewards. As in the other reforestation species studied, *Xylocopa* sp. and other large bees were aggresive and hostile to other visitors. Once free of other visitors, *Xylocopa* sp. inserted its mouthparts in the corolla opening and probed for the nectar located at the base of the ovary (Fig. 4). The flower of *P. indicus* is specialized and has an important functional feature. The pressure and/or weight of the pollinating vector as it alights at the wing petals cause the keel petal to expose the stamens and pistil previously covered and protected. Once freed, stamens and pistil touched with the ventral part of the head and thoracic region of the pollinating vector in a nototribic mode of pollination. Once pressure is applied to the wing petal the keel moves and is easily detached. After nectar had been consumed, the pollinator moved quickly to another panicle and/or flower or to a nearby tree. Nectar extraction usually took about 3 to 5 up to 7 seconds. Smaller bees apparently had problems in nectar extraction but succeeded in extracting pollen as shown in Table 5.

The average visit of the floral visitors per panicle is presented in Table 6. Average visit for all the visitors apparently did not differ much within the 2-week observation period except for *Apis cerana* Fab. and *Nomia* sp. posting 0.7 visit per panicle per hour.

Table 6. Average visit of floral visitor panicle/hour in *P. indicus*.

Floral visitors	Total number of visits from 0700–1800H	Average visit/ panicle/hour
Vespidae-1	30	4.2
Vespidae-2	10	1.4
Apidae: Apis2 cerana Fab.	5	0.7
Tachinidae	25	3.6
Halictidae: Nomia sp.	5	0.7
Apidae: Apis dorsata	20	2.8
Syrpidae	18	2.5
*Cicadellidae**	11	1.5
Xylocopidae: Xylocopa sp.**	21	3.0

SUMMARY AND CONCLUSION

The flowers of the four reforestation species studied are classified as bee flowers. They are characterized by production of scent, production of floral nectar (*A. auriculiformis, P. indicus* and *V. parviflora*) in different floral parts, yellow color (*A. auriculiformis, L. leucocephala* and *P. indicus*), open and easily manipulated by floral visitors (*A. auriculiformis, L. leucocephala* and *V. parviflora*). The extrafloral nectar of *A. auriculiformis* are produced in newly developed shoots/phyllodes and serves as food for ants which in turn offer protection against herbivory.

A. auriculiformis and *L. leucocephala* are pollen sources of floral visitors while *P. indicus* and *V. parviflora* are nectar sources. *A. auriculiformis* is pollinated by a single species of medium-sized bee (*Megachile* sp.). The limited number of pollinator of this species is attributed to its recent introduction. At present there exists an *A. auriculiformis-Megachile* sp. association in Mt. Makiling. The former depends on the latter for pollination and consequently fruit and seed production. The association may be exclusive and important to the succesful adaptation and naturalization of *A. auriculiformis* in the country. However, further studies along this line is needed to be conclusive. *V. parviflora* exhibited general mellitophylly. It is pollinated by several large to medium-sized bees notably, *Xylocopa* sp.

L. leucocephala has two floral visitors. *P. indicus* has specialized flower and each part performs definite function. Further, the flower is the functional unit requiring some tripping mechanism prior to floral reward extraction by several large to medium-sized bees notably *Xylocopa* sp. This mechanism promotes a close association between the flower and visitors. It may also encourage greater pollen and nectar economy.

Xylocopa sp. is an important pollinator of three of the species studied. Its population could affect the amount of seed and fruit production not only of species studied but other species of trees in Mt. Makiling as well.

ACKNOWLEDGMENT

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Fungi Toxic Effect of Some Medicinal Plants (On Some Fruit Pathogens)

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ABSTRACT

In present studies the systematic screening of medicinal plants were carried out and their fungitoxic principles had been determined. The different concentration of leaf extracts of medicinal plants were tested upon the spore germination of few fruit pathogens viz. Alternaria alternata (Fr.) Keissler, Dreschlera halodes and Helminthosporium speciferum.

INTRODUCTION

Since the beginning of the antibiotic era, a large number of antibiotics have been discovered from micro-organisms. Surprisingly the antibiotic principle of higher plants have received relatively little attention. Recent researches on fungitoxicity of higher plants have indicated their fruitful exploitation on a possible source of antifungal substances (Amer *et al.*1980; Bhowmick *et al.* 1981; Dubey *et al.* 1983). Considering the view, the present studies have been based on the antifungal activity of leaf extract of few selected medicinal plants viz. *Allium cepa* L.-Onion-Alliaceae, *Argemone mexicana* L.-Mexican prickly poppy-Papaveraceae, *Calotropis procera* R.Br.-Ivory plant, crown flower - Asclepiadacaeae, *Datura metel* L.-Hindu datura-Solanaceae (syn. *D. fastuosa* L., *D. Cornucopia* Hort.), *Ocimum sanctum* L.-Holy basil-Labiatae/Lamiaceae, *Rauvolfia serpentina* (L) Benth. ex Kurz.-Apocynaceae, *Tagetes erecta* L.-Marigold-Compositae/Asteraceae and *Catharanthus roseus* (L) G.Don-Madagscar periwinkle-Apocynaceae (syn. *Vinca rosea* L., *Lochnera rosea* Reichb.); on some fruit pathogens.

MATERIALS AND METHODS

The pathogens were isolated from diseased skin samples and were inocculated aseptically on PDA (Potato Dextrose Agar) medium (Ricker & Ricker, 1936) in-vitro. The selected pathogens were *Alternaria alternata* (Fr.) Keissler isolated from *Malus sylvestris* Mill.-Apple-Rosaceae, *Dreschlera halodes* from *Cucurbita moschata* Duch.-Pumpkin-Cucubitaceae and *Helminthosporium speciferum* isolated from *Zizyphus jujuba* Mill.-Jujube-Rhamnaceae; respectively. The isolated pathogens were grown on culture media and pure culture of these fungi were maintained.

The leaf extract of selected medicinal plants (*Allium Cepa* L., *Argemone mexicana* L., *Calotropis procera* R.Br., *Datura metel* L., *Ocimum sanctum* L., *Rauvolfia serpentina* (L) Benth. ex Kurz., *Tagetes erecta* L. and *Catharanthus roseus* (L) G.Don) were prepared separately by crushing with the help of pestle and mortar with a small amount of sand which facilitated the crushing and each leaf extract were prepared in five dilutions viz. 20, 40, 60, 80 and 100.

The spores from each culture were taken and their percentage germination were calculated under different dilutions of each leaf extract by hanging drop technique of Hoffmann. Three replicates were taken for each treatment along with the control series of each in sterilized distilled water in place of extract. The percentage germination of spores in each case was recorded after an incubation of 8-12h at 25 ± 1^0 C.

RESULT

It was observed that there was a sharp decline in percentage germination of spores of *Alternaria* which reached up to 8.8% as the dilutions were increased in case of *Argemone mexicana. Rauvolfia serpentina* also showed antifungal activity and reducing the spore germination at higher concentrations. The extract of *Datura metel, Calotropis procera, Ocimum sanctum, Catharanthus roseus, Tagetes erecta* and *Allium cepa* also influenced the germination of spores gradually, but not so much effective as the extract of *Rauvolfia serpentina* and *Argemone mexicana* (Figure 1).

The extract of *Allium cepa, Argemone mexicana, Datura metel* could not check the spore germination of *Dreschlera* at lower dilutions. The extract of *Ocimum sanctum* showed almost constant spore germination at all the dilutions i.e. from lower to higher dilutions. *Argemone mexicana* slightly inhibited the spore germination at higher concentration i.e. up to 72.7%, whereas *Allium cepa* and *Datura metel* showed 58.3% and 51.7% spore germination at 100% of extract. *Calotropis procera* at lower dilution did not show any effect but as the dilutions increased, there was gradual inhibition of growth of germ tube which reached up to 20.0% at 100% of

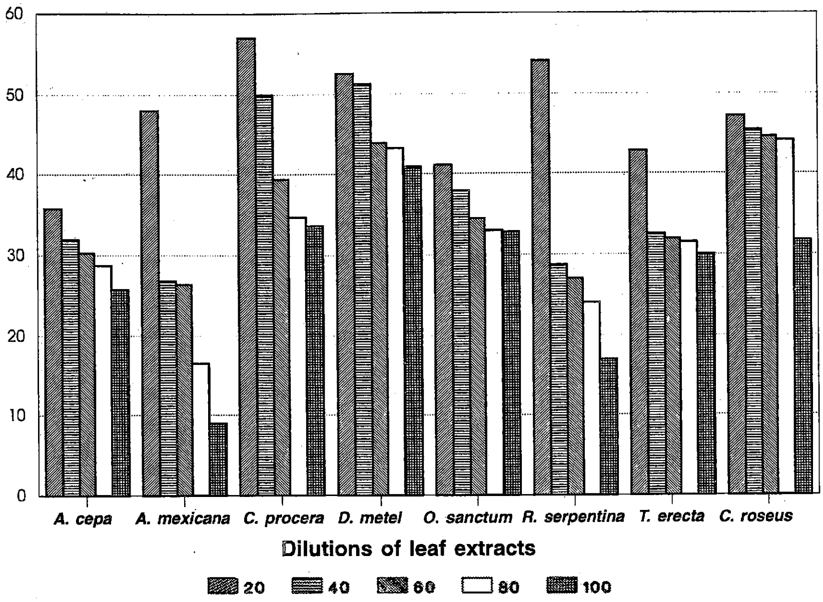

Figure 1. Percentage germination of spores of *Alternaria alternata.*

extract. There was a gradual decrease in percentage germination of spores in the increased concentrations of leaf extract of *Catharanthus roseus. Rauvolfia serpentina* was found best among all the medicinal plants studied for it checked spore germination in 20% of leaf extract which was about 42.1%, whereas at 100% of the extract, it became 5.1% (Figure 2).

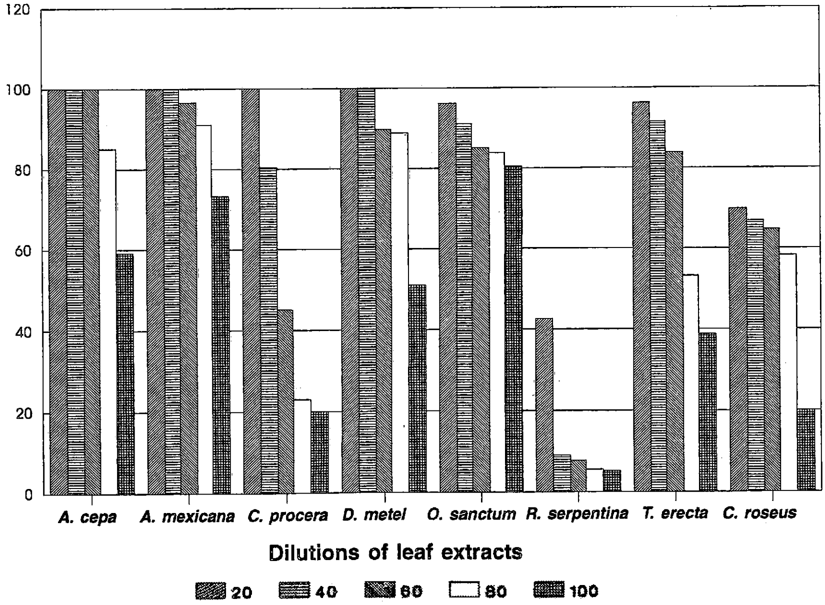

Figure 2. Percentage germination of spores of *Dreschlera halodes.*

All spores of *Helminthosporium* germinated in the leaf extract of *Argemone mexicana, Datura* metel and *Catharanthus roseus*. The extract of these plants could not inhibit the spore germination either in lowering the dilutions or while kept in higher dilutions. A slight decrease in germination was however, found in higher doses of extract of *Rauvolfia serpentina*. The extract of *Ocimum sanctum* showed gradual decrease in spore germination as the dilutions increased but became almost constant in 80% to 100% of leaf extract, which became 24.8%, whereas at lower doses the spore germination was recorded as 88.5%. The extract of *Tagetes erecta* reduced the spore germination at 20% and 40% of the extract, the percentage germination being 58.3% and 25.0%, but at higher doses i.e. from 60% and upwards, the spore germination became completely checked. In case of leaf extract of *Calotropis procera* at 20%, 11.1% of the spores germinated, while at higher doses none of the spores were found germinated (Figure 3).

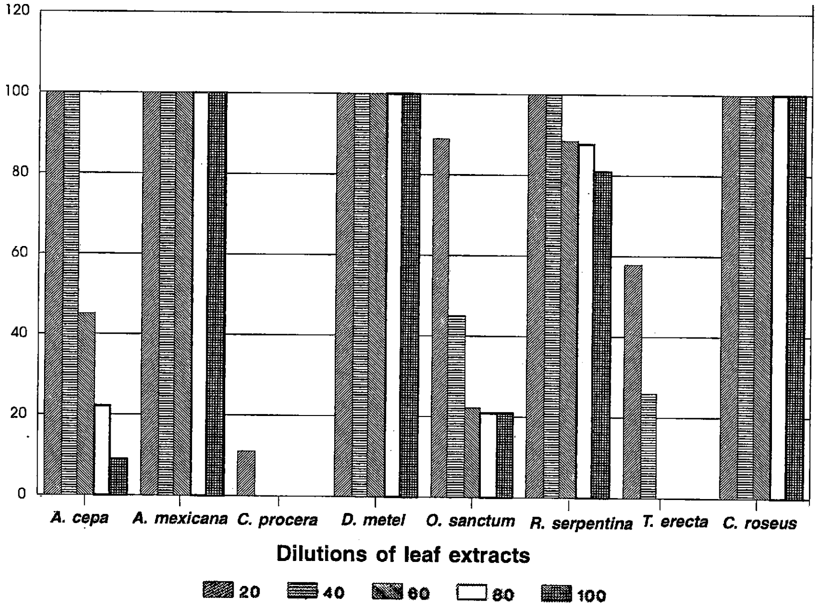

Figure 3. Percentage germination of spores of *Helminthosporium speciferum*.

DISCUSSION

The effect of some of the dilutions of certain medicinal plants were highly pronounced since complete inhibition was associated with them. All the drug yielding plants evaluated caused inhibition of spore germination. However, their action was pathogen specific. Some of them had a narrower range and were effective for only one pathogen whereas some had a very wide range and were effective against a number of pathogens tested e.g. *Rauvolfia serpentina*. The inhibitory action of plant extracts seems to be restricted to only certain concentrations falling within specific ranges. Mishra and Dixit (1977) screened out the antifungal activity of 47 angiosperms against *Alternaria alternata, Curvularia lunatus, Fusarium nivale* and *Helminthosporium gravineum* and concluded that the extract of some plants caused complete or partial inhibition while some induced the malformation and others stimulate the spore germination. Felklovia (1976) studied pathophysiological properties of some medicinal plants on some diseases and found that there was a decrease in Codeine and Baine contents in *Opium* infected by *Alternaria alternata.* Agarwal (1978) and Amer *et al.* (1980) reported that aqueous root or bulb extract of garlic sterilized by filteration reduced the linear growth rate of some rhizosphere and rhizoplane fungi more than did similar extracts from onion i.e. extracts of garlic was more stimulatory than those of onion. The characterstics of *Catharanthus roseus* extracts from leaves, flowers, stem and root showed antifungal activity against number of fungi like *Sclerotium, Pestalotia, Aspergillus niger* etc. By Narain and Satapathy (1978). Jetti *et al.* (1986) tested antifungal spectrum of leaf extract of *Polyalthia longifolia* Benth. & Hook. f.-Indian tree-Annonaceae and found its activity against some fungi to be in-vitro. Dey and Choudhuri (1984), Saksena and Tripathi (1985), Asthana *et al.* (1986) reported that extracts from leaves of *Ocimum* were more fungitoxic and showed antimicrobial activity than those from other plant parts such as inflorescence, stem and roots. The essential oil contents of leaf, inflorescence, stem and root were 0.97%, 0.28%, 0.09% and nil respectively. Therefore leaf extract showed more active effect against pathogens. These studies concluded that the chief components of oil which showed considerable activity against some crop pathogens are Eugenol and Methyleugenol. The leaf extract of *Lawsonia inermis* L. Henna-Lythraceae was also found antifungal on *Dreschlera oryzae* (Natrajan and Lalitha Kumari 1987). Other workers who also gave reports about fungitoxicity of higher plants especially medicinal plants are Bhowmick *et al.* (1981), Dubey *et al.* (1983), Nanir and Kadu (1987).

Since the spore germination is a combined effect of number of activities, its inhibition is expected to be achieved in number of ways each involving suppression of specific set of activities. Probably some of the plants inhibiting the spore germination possess more than one mode of action. The plant extracts seemingly caused reduction in mycelial yield and sporulation of all pathogens but to varying extents. In many cases the medicinal plants suppressed both growth and sporulation but in many others the suppression was confined to either growth or sporulation.

CONCLUSION

Though all the leaf extracts of selected medicinal plants were found to be effective against the spore germination of *Alternaria,* however, the extract of *Argemone mexicana* was found to give the maximum check on spore germination whereas *Rauvolfia serpentina* also reduced the spore germination up to a slight extent.

The spore germination of *Dreschlera* showed minimum percentage in almost all dilutions of *Rauvolfia serpentina* whereas *Calotropis procera* and *Catharanthus roseus* also reduced the percentage of spore germation at higher doses.

There was no effect on spore germination of *Helminthosporium* in *Argemone mexicana, Datura metel* and *Catharanthus roseus. Rauvolfia serpentiva* could not totaly inhibit the spore germination but could reduce the extent of their germination. The extracts of *Ocimum sanctum* and *Allium cepa* were found to reduce the percentage of spore germination at higher doses. The lower doses of *Tagetes erecta* could completely check the spore germination at higher doses but some of the spores germinated under lower doses. *Calotropis procera* was found best among all in inhibiting the spore germination from lower to higher doses. There was no spore germinaiton in the leaf extracts of *Calotropis procera* between the dilutions 40% to 100%.

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Studies on the Mycorrhizal Association of the Ornamental Orchid, Papilonanthe subulata (J. Koenig) Garay

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ABSTRACT

Orchid mycorrhizae are a category of endomycorrhizae. These are characterized by the presence of pelotons, which are balls of fungal hyphae in the cortical cells of the infected root. Each cell containing pelotonic and non-pelotonic hyphae, the former participating the digestion and the later organize the fresh peloton once the former is digested. These two types of hyphae plays a different roles in the association. The cytochemical and anatomical features of the mycorrhizal fungus associated with the epiphytic orchid, Papilionanthe subulata are described and discussed.

Keywords: peloton, formation, epiphytic orchid

INTRODUCTION

Orchids are a unique monophyletic group of plants that exhibit a number of relationships with specific group of fungi is termed as Mycorrhizas (Curra and Sherburna, 1992). Mycorrhizas are most important of all mutualistic symbiosis known to us (Peterson and Farquhar, 1994), also one of the poorly understood during the establishment of the symbiosis (Bonofante - Fasola and Perotto, 1991). Among the different types of mycorrhizas, orchids have a special type of intracellular hyphal coils in the cortical cells called pelotons.

Mycorrhizal fungi influence the growth of terrestrial orchids originally brought to attention by Bernard (1903) is now well established and subject has been revived frequently (Peterson and Currah 1990; Richardson *et al* 1992). In orchids, mycorrhizas are observed in at least part of the life cycle of the plant. In some, it may be found for short periods of root growth (Hadley 1982), While others such as *Rhizanthelia gardneri* R. Rogers the association is essential for the entire life cycle (Warcup 1992).

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The recent observations suggest that mycorrhizal fungi often do not colonise epiphytes (Lesica and Antibus 1990). Only in recent years there is a renewed interest in mycorrhizal infection in orchid roots. In India approximately 2,100 species (both terrestrial and epiphytic) of orchids have so far been reported (Sathish Kumar 1986). The widespread occurrence of infection has led to the general assumption that mycorrhiza have a function in epiphytic orchid also but until this has not been investigated in the Indian epiphytic orchids. However, much more basic information on the mycorrhizal status of epiphytic orchid is needed before generalization can be made about epiphytic dependence of the mycorrhizal fungi. This study, therefore, have been undertaken to investigate the process of mycorrhizal formation in the ornamental epiphytic orchid *Papilionanthe subulata (J.* Koenig) Garay.

MATERIALS AND METHODS

Epiphytic orchid *Papilionanthe subulata (J.* Koenig) Garay was collected from the Kolli hills (1,500 MSL), Salem district, South India. Its underground pseudobulbs produce several roots which have a mycorrhizal association. The involved fungus was identified as *Rhizoctonia repens* renamed by (Moore 1987). The roots were separated from the pseudobulbs and washed thoroughly in water and cut into small bits before fixing in Farmalin Acetic Alcohol (FAA). They were subjected to customary methods of fresh free hand sections as well as cryosections were also made. Both transverse and longitudinal sections were cut and were subjected to several cytochemical and cytoenzymological staining procedures (Table 1). Infection density and digestion percentage were calculated as per the formula provided by Hadley and Williamson (1972). For culculating this 50 sections were randomly selected and used. The width of the infected and uninfected cells from randomly selected sections were also measured (Fig. 1 & 2) separately to calculate the cell area (Katiyar, *et. al.* 1985). Photographs were taken using Nikon FA 35A Labophot (Japan) binocular research microscope.

Table 1. Cytochemical and cytoenzymological procedures..

S. No.	Procedures employed and treatment time	Substance to be localized, color to be obtained	Rationale
1	Acid fuchsin method (1% in distilled water)	Proteins-Reddish pink (Robinow and Merck 1966)	The aldehydes of proteins react with acid fuchsin and get stained
2	Aniline blue (aqueous solution of aniline blue 0.005%)	Callose - blue	The active component of the dye involved in the specific binding to callose specifically with the surface of 1,3-β Glucan trible helix possibly through hydrophobic regions
3	Methyl green (0.15% methyl green in 100ml of acetate buffer pH-4.7, 1% n-butanol 1-2 min)	DNA-blue (Gahan 1984)	Specific ionic binding of the basic dye methyl green to the acidic DNA molecules (Pearse 1985)
4	Toluidine blue O (0.05% in acetate buffer, pH 4.4)	Carboxylated polysaccharides-pink	TBO- A meta chromatic dye highly polymersed lignin shows orthochromatic blue
5	Esterase E.C. 3.1.1.1	color: Reddish brown	simultaneous azocoupling (Pearse 1985)
6	Peroxidase E.C. 1.11.1.7	color: Brown	simultaneous azocoupling azocoupling method (Dejong et al 1967)

RESULTS AND DISCUSSION

Infection was noticed in the inner most cortical cells, followed by infection in the more and more outer cortical cells. Sclerotia are noticed on the surface of the root, it has been observed that entry into the host root may be through velamen. At the point of entry no appresorial structure could be seen in the hyphae. Probably the fungus be used enzymatic dissolution of the root hair wall for its entry into root is always through root hairs and velamen. However, Senthilkumar and Krishnamurthy (1998) had observed that in *Spattlioglotis Plata* Blume entry is always through the root hairs.

The fungal hyphae may traverse various layers of velamen before entering into exodermal passage cells. From velamen onwards the hypha forming intracellular coils or pelotons followed by exodermal passage cells and finally settle down in the cortical parenchyma cells (Figure 1). Colonization of velamen and exodermal passage cells by fungal pelotons was observed first time here (Figure 2). It is likely that these cells may have the appropriate physiological condition for coil formation and digestion. The only instance of colonization of epidermal cells by the fungus is reported in *Goodyera repens* (L.) R. Br., by Peterson and Currah (1990), that this is also in the protocorm of orchid. The entry into the cortex from the rhizodermal layer is through passage cells of exodermis. This is noticed by several other investigators earlier (Esnault *et at* 1984; Senthilkumar and Krishnamurthy 1998).

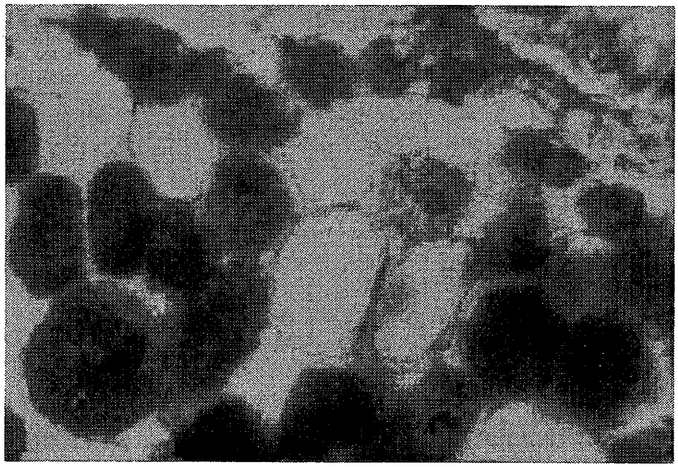

Figure 1. C.S of root stained with aniline blue shows the fungal coils. x150.

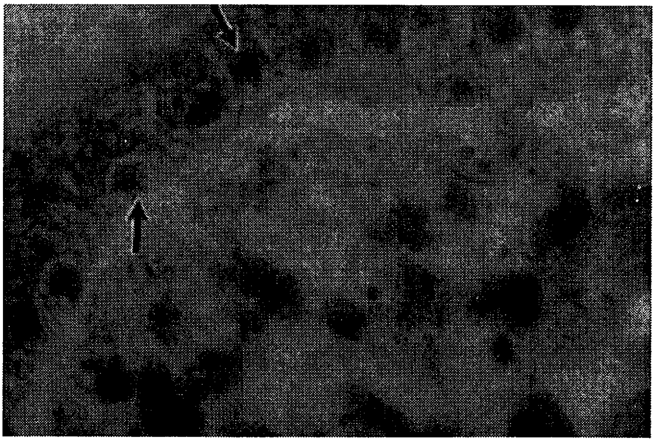

Figure 2. Fungal pelotons are seen in the velamen cells. (arrow) x150.

In Papilionanthe subulata showed an infection density of 30% to 85.7% with a mean of 63.8% and digestion was 31.9%. Katiyar *et al* (1985) studied 12 sps. of terrestrial orchids of northeastern India and recorded an infection density of 68.9% to 97% depending upon the orchid. Variation of the fungal colonization in epiphytic orchids may be the endophytic specificity, seasonal changes, host defense mechanism and etc. Benzing (1982) also reported that all roots which are in contact with the substrate were infected in an epiphytic orchid Florida. The present study were carried out for one time that infection was seen almost all the roots, but each of this roots, a small segments was not colonized by the fungus. Abiotic factors such as water and nutrient availability may also influence the mycorrhizal colonization. Epiphytes with many root hairs, however seem to be less dependent on mycorrhizal reported by Michelson (1983).

As in many other previously studied orchids, fungus was restricted only the cortex. Penetration of hyphae into endodermis was observed by Ruinen (1953) in a species of *Dentrobium nanum* Hook. F. was never observed in the present studied orchid. However colonization could be seen up to that layer of cortex adjoining the endodermis. To calculate the cell area infected and uninfected cells were measured. The area of the infected cell in general increased. Katiyar et. al. (1985), recorded some terrestial orchids that the uninfected ones did not increased.

As in many other previously studied orchids, fungus was restricted only in the cortex. Penetration of hyphae into endodermis was observed by Ruinen (1953) in a species of *Dentrobium nanum* Hook. F. was never observed in the present studied orchid. However colonization could be seen up to that layer of cortex adjoining the endodermis. To calculate the cell area, infected and uninfected cells were measured. The area of the infected cell in general increased. Katiyar *et al* (1985), recorded some terrestrial orchids that the uninfected ones did not increased.

Pelotons are loosely arranged fungal mycelial networks inside the cortex. In cross sections of all the species appear as spherical ball of mycelia. These are always formed in the certical cells of the roots and in the idioblast (Figure 3). One of the most striking events in the orchid mycorrhizal system is the lysis of the fungal coils (Peterson and Currah 1990). These coils were lysis of the fungal pelot6ns takes place, as already stated as 'digestion alls" by Burgeff (1959). The oldest colonized cells were act as the first digestion cells and followed by subsequent colonization. Purves and Hadley (1975), observed digestion after 48 hours of infection. But their present study, could not observe digestion after infection and significantly notified that digestion occured throughout the year in sequence from first infected to subsequently infected cells. Digestion was randomly observed in all the species, not in a defined zone of all, designated as the "digestion layers" in some species in the inner cortex (Hadley and Williamson 1970; Hadley 1982).

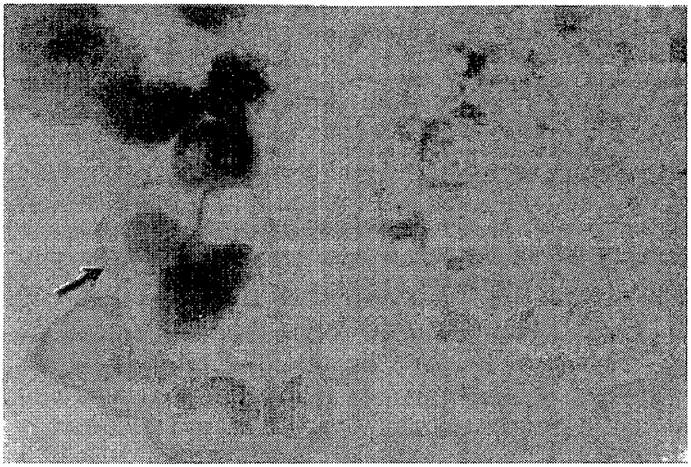

Figure 3. Fungal pelotons are restricted only in the cortex occasionally pelotons may be seen in the idioblastic (arrow) cells of the cortex. x150.

Separation of lysing pelotons from the rest of the host cell cytoplasm by a distinct layer of material designated as "cellulose slime layer" (Nieuwdrop 1972) suggest that this layer was callose. Callose was induced as a wound response to isolate the degenerating hyphae and presumably a high concentration of a lytic enzymes from the host cell cytoplasm. Here the similar layer was noticed to aniline blue around the lysing pelotons. Probably this formation was to effectively isolate and insulate the lysing pelotons from the host cell cytoplasm (Krishnamurthy 1977).

The infected root cortex reveals two types of hyphae which are often present, one forming pelotons and the other not participating in peloton organization. The non-pelotonic hyphae are observed around the cell wall of the host cell. Details of peloton formation in the same host cell has been reported recently by Senthilkumar and Krishnamurthy (1998). They further reported that the same host cell containing pelotonic and non-pelotonic hyphae in the former forming bundles and the later forming a layer around the host cell, is being connected by cross connections, and also the difference between these two types of hyphae have recorded in the terrestrial orchid *Spathoglottis plicata* Blume (Senthilkumar and Krishnamurthy 1998). But the details of renewed peloton formation in the same host cell have not been worked out in the past in any epiphytic orchid. The present study indicates the method by which successive pelotons may be organized.

The hyphae of the fresh pelotons are fully filled with cytoplasm and nuclei could be seen, the cytoplam is rich in carboxylated polysaccharides, total proteins and a fairly good amount of total lipids. A fairly high activity of peroxidase and esterase could be detected in the young hyphae. A gradual loss of these enzymes on the hyphal cell walls was also noticed during digestion.

There are a few noticeable events were detected in the host cell consequent to peloton formation. One of the striking event is the change in the host cell nucleus in their size. An instance of distinctly lobed nucleus is shown in Figure 5. Its affinity for toluidine blue 0, methyl green and acid fuchsin increase steadily. The nuclear changes in the peloton containing host cell have been reported earlier (Williamson 1970; Wemer 1992; Senthilkumar and Krishnamurthy 1998). The enzymes required for the lysis of the hyphae are produced by the host cell, as has been claimed by Burgeff (1959)., However it is more likely that the host cell controls the digestive process rather than the fungus. The present study recorded that there is a moderate activity of peroxidase and esterase in the fresh pelotons and this could not be detected in the lysed pelotons. Senthilkumar and Krishnamurthy (1998) reported in *Spathoglottis plicata* Blume that the peroxidase and esterase could not be detected in the younger pelotons. However, during lysis of pelotons and the activity of peroxidase increased while esterase could not be detected.

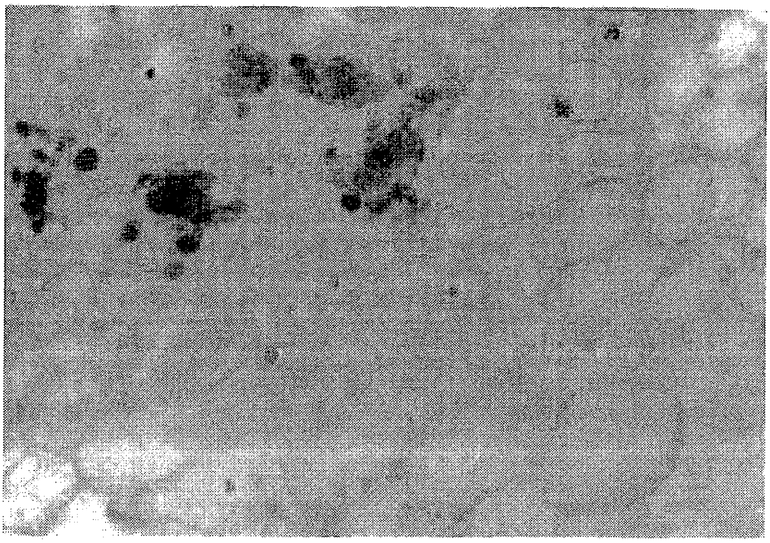

Figure 5. Note the prominent host cell nucleus than the uninfected one. x650.

When the digestion is going on in the original peloton, a fresh peloton is organizing in the same host cell (Figure 4). It is so rapid that two generation of the peloton could be seen in the same host cells of *Papilonanthe subulata (J.* Koenig) Garay. A number of workers have correlated such reinfection with periods of active growth of the orchid that depends on the root and the availability of the fungus.

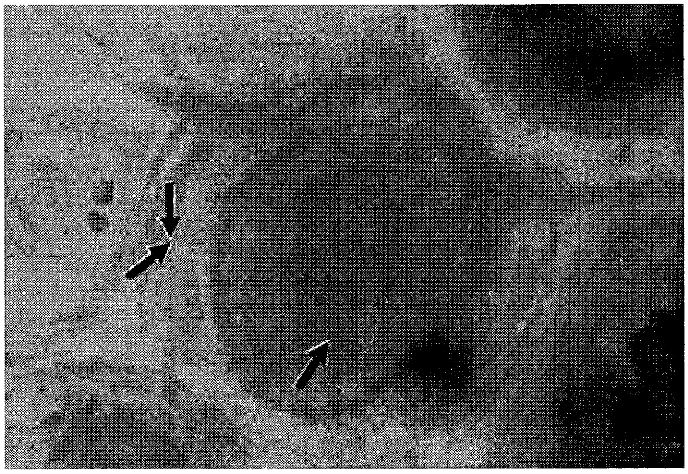

Figure 4. C.S of root cortex showing pelotons successively formed. The older pelotons in the same host cell are brownish in color (arrow) and are separated from the fresh pelotons (double arrow) by an aniline blue positive. Note the prominent host cell nucleus from the uninfected one. x150.

Details of the successful pelotons formations in the same cell have not been worked in the past in any epiphytic orchid. The present study indicates the method by which successive peloton formation may be organized. The non-pelotonic hyphae are separated from the pelotonic hyphae by the significant presence of phenol in the walls of these non-pelotonic hyphae which help them in organizing a fresh peloton in the same host cells this may be one of the reason that mycorrhizal cells have higher amounts of phenols compared to non-mycorrhizal cells.

One observation made in the present study is the role of root hairs. They are involved as the entry points for the fungus but also provide the venues for the reproduction (Figure 6). Chlamydospores are formed in plenty within the root hairs terminally and such chlamydospores containing root hairs undergo spiral dehiscence to release the spores into the rhizosphere.

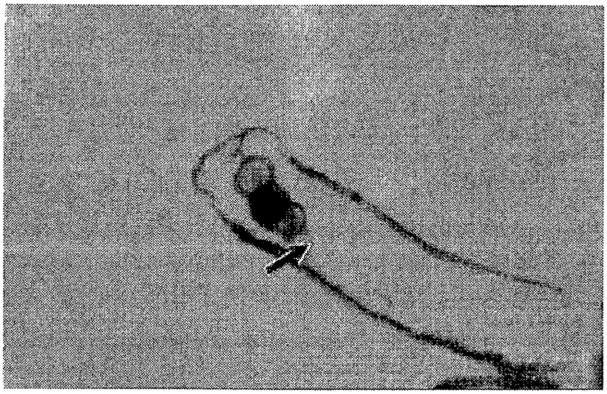

Figure 6. Chain of chlamydospores attached with fungal hyphae (arrow). x650.

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The Photocatalytic Reduction of Carbon Dioxide in Presence of Pyronine-B Coated Titanium Dioxide

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ABSTRACT

Photocatalytic reduction of carbon dioxide in aqueous media has been carried out in the presence of Pyronine-B coated titanium dioxide powder. The formaldehyde and formic acid were identified as photocatalytic reduction products and their amount were measured spectrophotometrically using Nash reagent. The effect of the variation of different parameters like amount of photocatalyst [Pyronine-B coated titanium dioxide powder], Pyronine-B concentration, light intensity, particle size etc., on the yield of photocatalytic reduction products was also investigated. A tentative mechanism for this reduction reaction has been proposed.

$$CO_2 \xrightarrow[H_2O]{\text{Pyronine-B Coated } TiO_2 + h\nu} HCOOH + HCHO$$

Key Words : Photocatalysis, Carbon dioxide, Pyronine-B, Titanium dioxide.

INTRODUCTION

The photochemical reactions Carbon dioxide and related compounds are of vital interest from an energy storage point of view and has attached the attention of many chemists[1-3] all over the world. However, little work has been done in this direction. Getoff[4,5] and Tanaka and Ozawa[6] were also able to produce formaldehyde and formic acid from the reduction of carbon dioxide. Later on this kind of work was carried out by different workers[7-10] . Inoue et. al[11]. Aurian Blajeni et. al[12] and Tanaguchi et. al[13] investigated the carbon dioxide reduction with an illuminated p-Gap electrode in suitable electrolytic solution. Kiwi et. al[14] reported the formation of formic acid as product in photoreduction of carbon dioxide using ruthenium bipyridyl complex, EDTA, colloidal TiO_2 and 1,1-terimethylene, 4,4-dimethyl-2-2-bipyridylium dibromide as photosensitizer, sacrificial electron donor, catalyst and strongly reducing electron relay, respectively, Hiroaki et al[15], Lehn et al[16,17] and Ogura et al[18,19] have also studied the photoreduction of carbon dioxide using various interesting systems. Yamamura et. al.[20,21] and Stalder et. al.[22] used the chemically derivatised electrodes for electrochemical reduction of aqueous bicarbonate to formate whereas Ameta et.al.[23], reported the photoreduction of methylene blue by aqueous bicarbonate.

Photocatalytic reduction of carbon dioxide using semiconductor material suspension has also been investigated by Dzhabiev and Uskov[24], and Kisch and Twardzic[25], Taqui Khan et.al.[26] used, [Ru(H-EDTA) Cl].$2H_2O$ system for the reduction of carbon dioxide to formate and formaldehyde in the presence of Pt-CdS-RuO_2, while Inoue et.al.[27] isocitrate dehydrogenase and CdS for the photochemical fixation of carbon dioxide and related compounds has been investigated by various workers; the conversion efficiency is relatively low.

A detailed survey of literature reveals that little attention has been paid to the use of dye coated semiconductor in aqueous media for the photocatalyic reduction of carbon dioxide and therefore, the present work was undertaken.

EXPERIMENTAL

Titanium dioxide (SdS) was used as a photocatalyst and it was coated with Pyronine-B (Stuttgart) in aqueous media to make it more sensitive in the visible region of the light. For this purpose, a solution of Pyronine-B of $5.0X10^{-5}M$ concentration was prepared in doubly distilled water and 1.0gm of titanium dioxide was added to 100.0 ml of this solution. This solution was allowed to stir for an hour for coating of dye and then it was filtered. After several washing, drying and activation, it was used as a photocatalyst.

One (1.0) gram of the Pyronine-B coated titanium dioxide was added to 150mL of water and carbon dioxide was then bubbled at the rate of 8.2mL / min. for 210 min. through the solution. The whole reaction mixture was exposed to light with a 500W tungsten lamp (Sylvania) [intensity $24.4mWcm^{-2}$]. The intensity of light was measured with the help of solarimeter [Suryamapi CEL 201]. A water filter was used to cut off the thermal radiation and low temperature was attained by a bath cooler [PBC-4-II, Neslab] to prevent evaporation of the photoreduced products.

Different samples were collected at different time intervals from this reaction mixture and the amount of formaldehyde and formic acid were estimated spectrophotometrically using Nash reagent[28] at λ_{max} 412 nm.

Nash reagent was prepared by adding 150gm of ammonium acetate, 3ml of glacial acetic acid and 2ml of redistilled acetyl acetone in doubly distilled water making its volume to 1 litre. This reagent was kept in dark so as to avoid any photochemical degradation.

For the measurement of formaldehyde concentration the test solution (0.5mL) was taken in a test tube and 2.0 ml of Nash reagent was added. This mixture was heated for 5 minutes in water bath at temperature around 50-60°C. A bright yellow coloured solution was developed. The solution was then allowed to cool at room temperature and then the optical density was measured at λ_{max} of 412nm. The concentration of formaldehyde in the test solution was determined from a calibration curve. The concentration of the formic acid was measured in an indirect manner using Nash reagent. First the formic acid was converted into formaldehyde by treating it with magnesium and hydrochloric acid[29].

Control experiment were also carried out in the absence of each component such as light, Pyronine-B, or TiO_2. It was observed that all the three components are necessary for the photoreduction of carbon dioxide. However with the use of uncoated TiO_2 in aqueous media the desired products were obtained but in much smaller yield.

The nitrogen gas was used to create an inert atmosphere so that no atmospheric carbon dioxide may participate or interfere in the reaction. It was observed that under this experimental conditions, atmospheric carbon dioxide does not interfere in the photoreduction.

RESULT AND DISCUSSION

The photocatalytic reduction of carbon dioxide was investigated using Pyronine-B coated titanium dioxide as photocatalyst in aqueous medium and the photoproducts, formaldehyde and formic acid, were estimated spectrophotometrically using Nash reagent (λ_{max} 412nm). A change in the yield of the photoproducts with the time of irradiation is reported in Table 1.

Table 1. Photogeneration of formaldehyde and formic acid.

[Pyronine-B] = 5.0 X 10^{-5}M
TiO_2 = 1.0 g

Temperature = 283 K
Intensity = 24.4mWcm^{-2}

TIME (Min)	[HCHO] X 10^4M	[HCOOH] X 10^4M
0	0.00	0.00
15	0.06	2.10
30	0.11	4.23
45	0.30	6.33
60	0.42	7.81
75	0.49	9.15
90	1.10	9.94
105	2.02	10.60
120	2.88	9.32
135	4.23	7.23
150	5.81	4.21
165	7.96	2.55
180	9.01	1.20
195	9.08	1.16
210	9.01	1.20

Initially, an increase in the yield of formic acid was observed and at 105 minutes, maximum yield was achieved. Then there was a decrease in its yield which shows its conversion into formaldehyde. The maximum yield of formaldehyde was achieved in 195 minutes.

Effect of Variation of Pyronine-B Concentration

The effect of variation of pyronine-B concentration on the yields of formaldehyde and formic acid was also observed. The results are given in Table 2.

Table 2. Effect of Pyronine-B concentration on the yields of HCHO and HCOOH.

Time = 195 min.
TiO_2 = 1.0 g
Temperature = 283 K
Intensity = 24.4mWcm^{-2}

[Pyronine-B] x 10^5M	[HCHO] X 10^4M	[HCOOH] X 10^4M
1.0	0.67	1.22
2.0	2.19	3.51
3.0	3.85	6.49
4.0	6.67	8.85
5.0	9.01	10.60
6.0	6.23	9.58
7.0	4.15	7.47
8.0	2.18	4.52
9.0	1.23	2.46

It is clear from Table 2 that there is an increase in the yield of formaldehyde and formic acid both with an increase in the Pyronine-B concentration and a maximum is reached at concentration 5.0 X 10^{-5}M. Further, an increase in concentration resulted in a decrease in the yield of HCHO and HCOOH. It may be due to the fact that as the concentration of Pyronine-B was increased, more dye molecules were available for excitation and energy transfer. It has resulted into a corresponding increase in the amount of formic acid and formaldehyde. On reaching an optimum yield, further increase in concentration may form a multi layer of Pyronine-B molecules near the semiconductor surface. Thus the energy transfer and/or electron transfer will be inhibited to a certain extant and in turn the amount of HCHO and HCOOH will decrease.

It was also observed that the higher concentration of Pyronine-B [more than 5.0 X 10^{-5}M] optimum values are attained within a smaller period of irradiation while longer concentration requires a longer period of irradiation (of Figure 1. 2). In both direction the yield decreases.

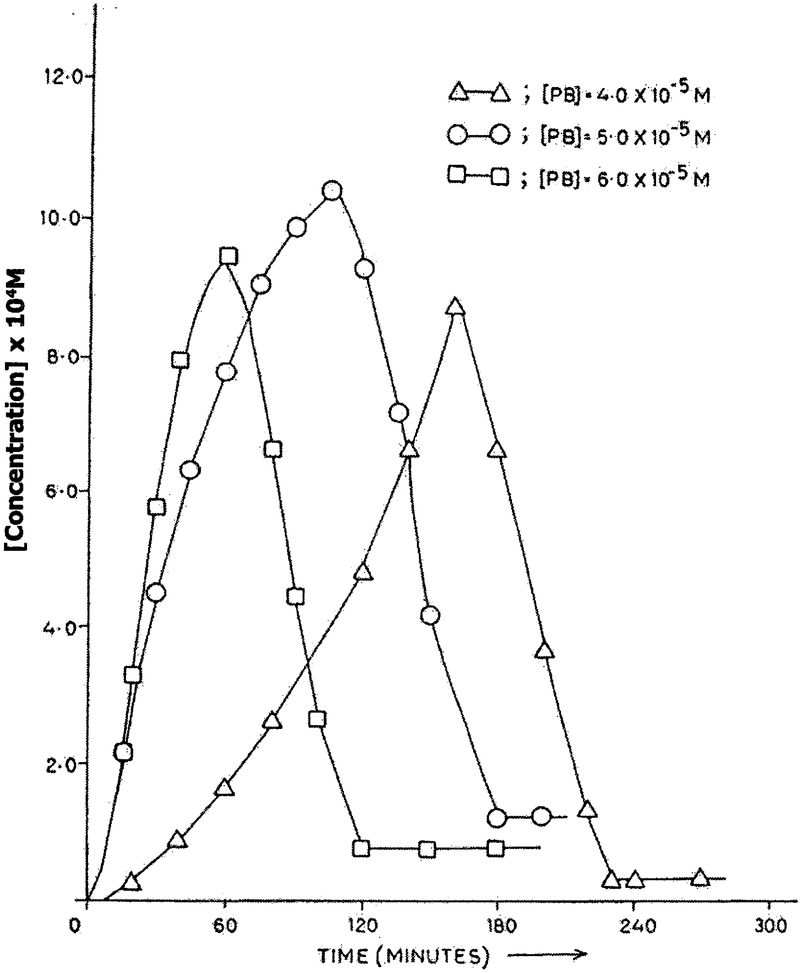

Figure 1. The effect of Pyronine-B concentration on production of formic acid.

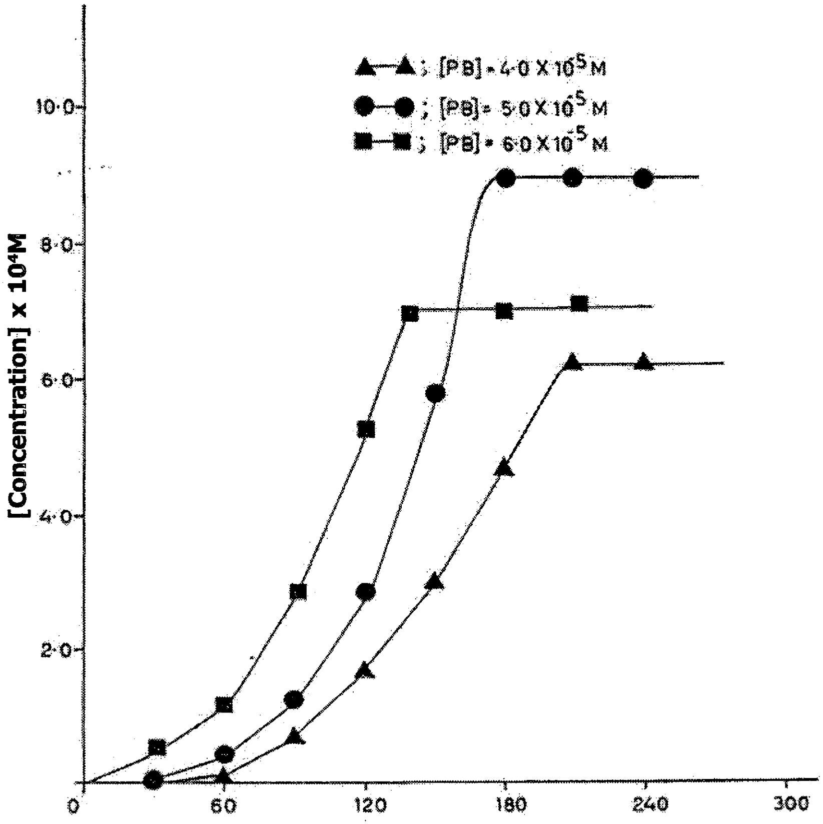

Figure 2. The effect of Pyronine-B concentration on production of formaldehyde.

This can be explained on the basis that more molecules of sensitizer [Pyronine-B] are available for participation in this reaction at its larger concentration, thus attaining optimum value in a shorter period while the reverse is true for lower concentrations of sensitizer [Pyronine-B].

Effect of Amount of Photocatalyst

The dependence of the yield of HCHO and HCOOH on the amount of Pyronine-B coated TiO_2 powder was studied and the results are summarised in Table 3.

Table 3. Effect of amount of photocatalyst.

[Pyronine-B] = 5.0×10^{-5}M
Time= 195 min

Temperature = 283 K
Intensity = 24.4mWcm^{-2}

Photocatalyst (g)	[HCHO] X 10^{4}M	[HCOOH] X 10^{4}M
0.20	1.42	3.81
0.50	5.09	7.64
0.80	7.72	9.92
1.00	9.01	10.60
1.20	9.04	10.60
1.50	9.01	10.61
1.80	9.08	10.60
2.00	9.06	10.57
2.50	9.00	10.60
3.00	9.01	10.60

An increase in the yields of both HCHO and HCOOH was observed with the increase in the amount of photocatalyst. The yield of the products attained optimum value for 1.0g of photocatalyst. No further increase in the yields of photoproducts was observed and a plateau was obtained.

This may be attributed to the exposed surface area of the reaction vessel which is completely covered by a specific number of sensitized TiO2 particles and further increase will not increase the number of semiconducting particles exposed to the light. Therefore it may be concluded that this limiting value will depend upon the geometry of the reaction vessel. This was confirmed in an experiment with sensitized TiO2, where reaction vessel of different dimensions were used. It was observed that this limiting value was shifted to a higher range for larger vessels whereas a decrease in limiting value was observed for smaller vessels. Bigger vessels have wider area exposed to light. Thus more particles (TiO2) are exposed to light.

Effect of Light Intensity

The effect of intensity of light on the formation of products (HCHO and HCOOH) of photocatalytic reduction of carbon dioxide was also investigated using sources of different intensities ranging between 10.4 - 33.2 mWcm^{-2}. The results are reported in Table 4.

Table 4. Effect of variation of light intensity.

[Pyronine-B] = 5.0×10^{-5}M Temperature = 283 K
TiO_2= 1.0 g Time = 195 min

Photocatalyst (g)	$\log_{10}I$	[HCHO] X 10^4M	[HCOOH] X 10^4M
10.4	1.0170	2.78	6.13
13.5	1.1301	5.03	7.62
15.6	1.1931	6.08	8.47
22.4	1.3500	8.30	10.24
24.4	1.3874	9.01	10.60
27.2	1.4370	9.90	11.10
30.8	1.4880	10.81	11.93
33.2	1.5210	11.34	12.39

An increase in intensity of light increases the number of photons striking per unit area of the Pyronine-B coated titanium dioxide powder. Therefore, one expects a direct linear behaviour between light intensity and the yields of formic acid and formaldehyde, but it was never observed. On the other hand, a logarithmic behaviour was observed. The light intensity of medium order (24.4 mWcm^{-2}) was used throughout the present investigation except for this intensity variation experiment.

It was also observed that increase in the intensity of light not only affects the yields of HCHO and HCOOH but it also affects the length of time needed to achieve optimum concentrations of these photoproducts. Larger intensity of light will increase the number of photons striking the sensitizer molecules and, therefore, optimum concentrations of formic acid and formaldehyde were obtained in a shorter period, while the reverse is true for lower intensity of light.

Effect of particle size

The effect of particle size of photocatalyst on the yields of photoproducts (HCHO & HCOOH) was also studied by taking titanium dioxide of different particle sizes coated with Pyronine-B. The results are summarized in Table 5.

Table 5. Effect of particle size.

[Pyronine-B] = 5.0×10^{-5}M
TiO_2 = 1.0 g
Temperature = 283 K
Time = 195 min
Intensity = 24.4mWcm^{-2}

Particle size (μm)	[HCHO] X 10^4M	[HCOOH] X 10^4M
0.8	9.01	10.60
1.6	8.36	9.34
2.4	6.59	7.12
3.2	4.88	6.82
4.0	3.98	6.08

It is clear from the table that smaller particles of the photocatalyst gave more yields of products as compared to the bigger particles. This may be explained on the basis of the increased exposed surface area of photocatalyst as the particle size was reduced. The surface area of the semiconductor will increase by 4 times as the particle size is reduced by half of its original value. This should increase the yield 4 times, but it was never achieved. This may be attributed to the fact that the surface area exposed to light source will not increase to the extent as expected theoretically.

The formic acid and formaldehyde can also be generated using unsensitized titanium dioxide as photocatalyst but the yield of these photoproducts were relatively low (HCHO = 2.80 X 10^{-4}M & HCOOH = 4.60X 10^{-5}M) as compared to the Pyronine-B coated titanium dioxide. Therefore, it may be concluded that Pyronine-B coated titanium dioxide is more sensitive as a photocatalyst for photoreduction of TiO_2.

The formic acid and formaldehyde both can be burnt in a fuel cell to generate electricity and the photocatalytic synthesis of these compounds will not only solve the problem of scarcity of fuel but their use will not add even a single molecule of carbon dioxide to pollute it further, as these are synthesiszed from carbon dioxide of the atmosphere.

MECHANISM

On the basis of the observed data, the following tentative mechanism may be proposed for the photocatalytic reduction of carbon dioxide using Pyronine-B coated titanium dioxide as photocatalyst.

$$CO_2 + H_2O \xrightarrow[h\nu]{\text{sensitized } TiO_2} HCOOH + HCHO$$

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Effects of Two Vertebrate Hormones on the Biology of Silkworm Bombyx mori. L

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ABSTRACT

Effects of pregnant mare serum gonadotropin (PMSG) and thyroxine hormones on the biology of silkworm, Bombyx mori. L was studied. Single dose of (5 μg/ml) of PMSG and thyroxine were topically applied at 48 hours of fifth instar in silkworm. Observations were made to know the effects of treatments on bioassay of various economic characters of silkworm like larval weight, total larval duration, cocoon weight, cocoon shell weight, pupal weight, cocoon shell ratio, filament length, denier, eclosion period and fecundity both in PMSG and thyroxine treated groups. The results indicated that the thyroxine showed improvement in filament length, shorter larval duration and early eclosion characters and PMSG showed improvement in high fecundity, shorter larval duration and early eclosion. The significance of PMSG and thyroxine in the development of silkworm with the concomitant appraisal of economic characters is discussed.

Keywords : Bombyx mori, PMSG, Thyroxine, Economic characters.

INTRODUCTION

The presence of vertebrate hormone like compounds in insects and crustaceans have been reported (De Loof, 1987, Lafont, 1991). Previous studies (Bharathi et al.. 1983; Bhaskar et al.. 1983; Bharathi and Govindappa, 1987) revealed that the vertebrate pituitary extract, prolactin and prostaglandins accelerated the growth of silkworm and advancement in the pupation with increased weights of cocoons. Magadum and Magadum (1993) studies the effects of testosterone propionate on the economic traits of silkworm. Similarly, there are reports on the effects of topical application of prolactin, thyroxine and insulin on silkgland, fat body and gonads (Magadum and Hooli, 1988; Venkatarami Reddy et al.. 1992). The effect of thyroxine on the development of quantitative characters and growth of silkworm was studied by (Majumdar and Medda, 1975 ; Narasimha murthy et al., 1987. Thyagaraja et al., 1991 and Zhang et al.. 1992). Hormones have been known to participate actively in the reproduction and other physiological activities of the vertebrates. Although the effects of thyroxine on silkworm with respect to improvement in the growth and advancement in the larval duration has been made by (Chandhuri and Medda, 1985, Chandhuri and Medda, 1985: Magadum and Magadum, 1993) but the mode of treatment in our study was different. Earlier, the effects were noted by oral feeding of silkworm through mulberry leaf. However this is an attempt to know the effects of PMSG and thyroxine on the economic characters through the topical application of these two hormones.

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MATERIALS AND METHODS

A total of 10 disease free layings (on average each laying consisted approximately 400 eggs) of Pure Mysore breed of silkworm (Bombyx mori. L were brushed en masse (Brushing : Silkworm eggs will be sticking to egg cards or allowed in loose form for subsequent use. Brushing is the process of separating the newly hatched larvae gently from the empty egg shells on to the rearing tray with the help of a soft brush). The silworms were reared under standard conditions of temperature: 23°C - 29°C; relative humidity: 70-85% and 12 : 12 h light:dark as per the method of Krishnaswami (1978). After the third moult/ecdysis the larvae were distributed into 5 holding trays to represent replications) by keeping 100 larva/replication. Pregnant mare serum gonadotropin (PMSG) was obtained from Invert Company, Holland, whereas thyroxine was obbtained in the form of tablets from Allenbury pharmaceuticals, Glaxo, Bombay. 100 μg of thyroxine was dissolved in 20 ml of distilled water and 2 - 3 drops of acetone. In this way a single dose of 5 μg thyroxine/ml was prepared and applied topically on each silkworm. Likewise 1 I.U. of of PMSG was dissolved in 5 ml distilled water to get the stock solution which was stored in refrigerator. From 1 ml of stock solution of PMSG (200 μg) a working solution 5 μg/ml was prepared by adding 39 ml of distilled water and 2 - 3 drops of acetone. In this way a single dose of 5 μg/ml was prepared and applied on each silk worm. The experiment was conducted at 48 hours on fifth instar larvae. In control group the larvae were maintained without any application. The observations were made on 10 economic characters namely larval weight, total larval duration, cocoon weight, cocoon shell weight, pupal weight, cocoon shell ration, filament length, denier, eclosion and fecundity. A 'P' value of less than 0.05 was taken as the criterion of significance. The results of the treatment groups were compared with the control group by using Student's "t" test.

RESULTS

Data on the effects of PMSG and thyroxine treatment on different economical characters is as follows:

Larval weight: Highly significant (P<0.001) increase was noticed in the larval weight after PMSG and thyroxine treatments. The increase was 10.06% and 13.58% respectively in PMSG and throxine treated silkworm larvae as compared to control (Table . 1).

Table 1. Effect of Effect of pregnant mare serum gonadotropin (PMSG) and thyroxine treatment on the economic characters of silkworm Bombyx mori. L. Each value represents the Mean of 25 observations. Mean ± S.D., ± indicate the per cent increase or decrease over control, respectively. 'p' denotes the level of significance.

Sl No.	Component		Control	PMSG treated	Thyroxine treated
1	Larval weight at V instar (mg)	Mean	1952.5	2149.0	2217.66
		S.D	± 14.72	± 16.88	± 17.26
		%Change		+ 10.006	+ 13.58
		't' test		P<0.001	P<0.001
2	Total larval duration (days)	Mean	28.80	26.5	26.0
		S.D	± 0.52	± 0.63	± 0.52
		%Change		– 5.35	– 7.14
		't' test		P<0.001	P<0.001
3	Cocoon weight (mg)	Mean	1155.17	1290.83	1316.00
		S.D	± 7.98	± 9.67	± 10.31
		%Change		+ 11.74	+ 13.92
		't' test		P<0.001	P<0.001
4	Cocoon shell weight (mg)	Mean	144.1	167.3	166.2
		S.D	± 8.31	± 9.92	± 9.19
		%Change		+ 16.09	+ 15.33
		't' test		P<0.001	P<0.001
5	Pupal weight (mg)	Mean	1011.17	1122.33	1149.83
		S.D	± 6.82	± 7.5	± 8.7
		%Change		+ 10.99	+ 13.71
		't' test		P<0.001	P<0.001
6	Cocoon shell ratio (%)	Mean	12.46	12.94	12.63
		%Change		+ 3.85	1.36
7	Filament length (Mts)	Mean	364.16	424.0	427.8
		S.D	± 10.55	± 16.63	± 15.83
		%Change		+ 16.43	+ 17.47
		't' test		P<0.001	P<0.001
8	Denier	Mean	2.3	2.36	2.44
		S.D	± 0.191	± 0.172	± 0.164
		%Change		– 7.0	– 8.3
		't' test		P<0.001	P<0.001
9	Eclosin (days)	Mean	10.0	9.0	9.0
		S.D	± 0.82	± 0.68	± 0.72
		%Change		– 10.0	– 10.0
		't' test		P<0.001	P<0.001
10	Fecundity	Mean	374.0	436.0	417.0
		S.D	± 10.44	± 8.16	± 8.29
		%Change		+ 16.57	+ 11.49
		't' test		P<0.001	P<0.001

Total larval duration: There was a highly significant (P<0.001) decrease in the larval duration after PMSG and thyroxine treatments. The decrease was 5.35 and 7.14% respectively in PMSG and thyroxine as compared to control (Table 1).

Cocoon weight: There was a highly significant (P<0.001) increase in cocoon weight after PMSG and thyroxine treatment. There was an improvement of 11.74% and 13.92% in cocoon weight after treatment when compared with control (Table 1).

Cocoon shell weight: Highly significant (P<0.001) increase was noted in cocoon shell weight after PMSG and thyroxine treatments. There was an increase of 16.09% with PMSG and 15.33% with thyroxine when compared with control (Table 1).

Pupal weight: Highly significant (P<0.001) increase was noted in pupal weight after PMSG and thyroxine treatments. The increase in pupal weight was 10.99% with PMSG and 13.71% with thyroxine over control (Table 1).

Cocoon shell ratio: Non-significant increase was noticed in cocoon shell ration in the PMSG and thyroxine treated silkworms. An increase of 3.85% and 1.36% in PMSG and thyroxine treated larvae was noticed when compared to control (Table 1).

Filament length: Highly significant increase (P<0.001) was noticed in the filament length after PMSG and thyroxine treatments. There was an increase of 16.43% and 17.47% in PMSG and thyroxine treated silkworms respectively when compared to control (Table 1).

Denier: There was a highly significant decrease (P<0.001) in denier after PNSG and thyroxine treatments. The decrease in the denier was 7.0% in PMSG and 8.3% in thyroxine after treatment when compared to control (Table 1).

Eclosion: Highly significant (P<0.001) decrease in the eclosion period was noted after PMSG and thyroxine treatments. The decrease in eclosion was about 10% in both PMSG and thyroxine treated silkworms over control (Table 1).

Fecundity: Highly significant increase was noticed in ficundity (P<0.001) thyroxine and PMSG treatment. The fecundity was improved by 16.57% and 11.49% in PMSG and thyroxine treated silkworms, respectively (Table 1).

DISCUSSION

The results of the present study clearly shows that all the economic characters of silkworm *Bombyx mori.* could be improved after PMSG and thyroxine treatments. Vertebrate hormones stimulate growth, lipid metabolism, sugar uptake and cellular internalization in insects and other invertebrates (Kramer, 1983) Recent studies have shown that treatments with vertebrate hormones improves pre-cocooning and some of post cocooning parameters of silkworm. (Bharathi et al., 1996; Magadum and Hooli, 1989). The vertebrate hormone namely prolactin induced improvement in the growth and reproductive potential of silkworm (Bhaskar et al., 1983 and Bharathi et al., 1984). Besides hormones some active principles like prostaglandins F2oC exhibited profound inmfluence on the growth rate, larval stages cycle and fecundity (Bharathi, 1993). There are reports showing that the economic characters of silkworm can be improved after the administration of PMSG (Rajasekhar, 1993) and thyroxine (Thyagaraja et al., 1991). It was reported that the vertebrate thyroid hormone treatment brings about various physiological changes in insects in general (Bhaktan and Gilbert, 1968 ; Novok, 1975) and the impact of thyroxine on the growth and metabolism of Bombyx mori in particular (Thyagraja et al., 1985; Magadam and Hooli, 1988; Venkatarami Reddy et al., 1992 ; Chaudhuri and Medda, 1992). An increased level of oxygen uptake and consumption occurs in invertebrates when treated with thyroid extracts (Ashbel, 1935). Srinivasan et al., (1955) and Bhaktan and Gilbert, (1968) have shown that thyroxine regulates the basal metabolic rate, thus accelerating growth and development. Thus the silkworms with the above two hormones were evaluated for 10 economic characters. The increase in larval weight, cocoon weight and cocoon shell weight shows the growth promoting effects of PMSG and thyroxine in *Bombyx mori.* This observation suggests that the hormone favours the metamorphic events in insects in general and even may influence the yield potential in silkworm in particular. (Mathias and Lucile, 1954; Srinivasan et al., 1955). Economic characters like cocoon shell weight, cocoon shell ratio, filament length, were increased and the denier value was decreased in the thyroxine and PMSG treated larvae. As the denier decreased the quality of silk filament was found to be superior which has much economic importance in the silk reeling industry (Sailaja et al., 1997). This simulating capacity of vertebrate hormones on the various characters contributing to silk yield may be attributed to the synthesis of proteins and nucleic acids in the silkworm. In view of the above observations it may be presumed that both these vertebrate hormones may stimulate that synthesis of lipids and/or steriods in silkworms by developing larval duration. The decrease in larval duration is also associated with the early pupation and growth promoting effect of thyroxine and PMSG (Thyagaraja et al. 1985). The advancement in the eclosion period and increase in fecundity is of practical use in silkworm egg production. In grainures/ commercial silkworm seed production centres the advancement in the eclosion presents the completion of work at an earliest, thereby saving both time and labour. Similarly sericulture farmers are always fascinated towards more number of eggs per laying, as this increases the cocoon yield. The higher lipid content in ovaries of PMSG and thyroxine treated larvae is indicative of mobilization of organic substances into the oocyte (Rajassekhar, 1993). It is a well known fact that hormones accelerates the vitellogenesis in insects which is due to increased function of topocytes in the germanium (Landa, 1970).

Thus, the mechanisms by which these two vertebrate hormones exerts their actions is still undertermined. It may be a general direct effect upon body cells, by regulation of the organs of internal secretation (endocrine glands) or both. Chaudhuri and Medda (1986) have reported that the effects of these two hormones are dependent on the time of administration and also on the developmental stages of the silkworm. It may be concluded from the present study that both PMSG and thyroxine hormones have certain effects on total larval duration cocoon weight, shell weight, pupal weight, shell ratio, filament length, denier, eclosion and fecundity. The application of these two vertebrate hormones is useful for sericulture in terms of economic importance. However, future detailed studies are required to find the mechanism of action of these two hormones in silkworm (*Bombyx mori.* L).

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